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(57) Abstract

The present invention relates to antigenic preparations comprising polysaccharides and/or glycopeptides preparable from keratinophilic fungi as well as yeasts, processes for the preparation of these antigenic preparations, their use as pharmaceutical substances as well as their use as vaccines, including but not limited to, the prophylaxis and treatment of allergy, as well as for modulating the immune response.

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Antigenic Preparations

The present invention relates to antigenic preparations comprising polysaccharides and/or glycopeptides preparable from keratinophilic fungi as well as yeasts, processes for the preparation of these antigenic preparations, their use as pharmaceutical substances as well as their use as vaccines, including but not limited to, the prophylaxis and treatment of allergy, as well as for modulating the immune response.

Allergy in one form or another afflicts more than 20 per cent of the human population, and the alarming increase in its prevalence, morbidity and mortality over the past decade has led to its designation as the number one environmental disease (Sutton and Gould, Nature 1993, 366, pp. 421-428). Human and animal populations are afflicted by allergy to a similar extent.

In the development of allergy, immunological reactions play a key role (Paul, William E. (Editor), Fundamental Immunology, Raven Press Books Ltd., New York, 1984). In principle two different types of allergic reactions have been described. One is immediate type hypersensitivity (ITH), for which the maximum allergic response to the allergen is observed within minutes to hours. The second is delayed type hypersensitivity (DTH). In case of DTH, the allergic response to the allergen usually reaches its maximum after 24 to 48 hours. Most likely ITH is mediated predominantly via the IgE pathway, whereas DTH is more complex. In the development of DTH it is likely that

further cell mediated responses (i.e. B- and T-lymphocytes) are involved. For example, after transferring lymphocytes and antibodies from allergic donor animals to non-allergic recipient animals, the recipients developed DTH (Askenase, P.W. (1973), J. exp. Med., 138, pp. 1144-1155).

Because of their direct exposure to environmental antigens, tissues most afflicted by allergies are the epithelial tissues, especially the skin. For example, in the dermatological clinic, acute allergic contact dermatitis and chronic allergic contact eczema account for up to 15% of all dermatoses. Allergic asthma accounts for about 20% of all asthma cases in humans.

Allergic diseases that can be classified as ITH, are for example atopic eczema, allergic bronchial asthma, hay fever, rhinitis, conjunctivitis. These can develop into chronic forms as well and should not be considered exclusively as IgE-dependent reactions. Examples of DTH are acute allergic contact dermatitis and chronic allergic contact eczema, which can further be classified as DTH (type IV) with epidermal involvement. Such a patient would have previously been sensitised through contact with an allergen and has developed hypersensitivity. Renewed contact with the allergen results in acute, sub-acute or chronic inflammatory contact dermatitis.

One example for an allergic dermatitis from the veterinary clinic is Summer Eczema, also called Sweet or Queens land Itch. Summer Eczema is an allergic dermatitis of horses, belonging to the atopic form of allergic diseases (involving Type I and IV reactions). Summer Eczema is provoked by the bite of midges of the

families Culicidae and Ceratopgonidae, and characterised by skin lesions with permanent erosions and exudations, mainly in regions of the mane, tail, and abdomen. Afflicted animals display a strong sensitivity of the skin with regard to irritations, i.e. touch, rain, wind etc., impairing their overall health and performance. As with other allergies, it is believed that the development of this disease is also influenced by nutritional factors. The symptoms of this disease are only visible from March to September, whereas the allergen induced sensitivity of the skin is observed during the whole year. Summer Eczema provides an interesting general model system for the study of allergy and for the development of anti-allergic substances.

Many treatments for allergy have been proposed, depending on the clinical picture. For the treatment of acute allergic contact dermatitis, chronic allergic contact eczema and/or atopic eczema usually lipophilic creams comprising glucocorticosteroids, anti-microbial substances, anti-inflammatory drugs and/or calcium are used. For the treatment of Summer Eczema various compounds have been applied locally or parenterally, for example steroid preparations, insecticides, different galenic formulations, salicylates, oils or peptides isolated from micro-organisms. All of the above treatments only deal with the symptoms and not the causes of allergy.

Impaired immune response or immunodeficiency often play important roles in the development of allergy. Therefore, also immunotherapeutic methods, for example the administration of immune-stimulators like BCG,

levamisol and other stimulators, have been used for the treatment of eczema, atopic eczema, skin abscesses, and also auto-immune diseases (A.M. Tschernucha (Editor), Koscha, published by Medicina in 1982, Moscow).

For the treatment of flea-allergic dermatitis, the administration of antibody derived peptides has been successfully used (British patent application No 8913737). For the treatment of atopic eczema, desensitivisation has also been used with relatively good results (A.M. Tschernucha (Editor), Koscha, published by Medicina in 1982, Moscow).

In spite of the various different approaches in treating allergy, to our knowledge, no antigenic compounds preparable from keratinophilic fungi or yeasts have been used for the treatment of allergy.

In the context of the present invention the term "soluble" or "nonsoluble" refers to the solubility in aqueous solution. The term "antigenic preparation" refers to any composition of matter that is able to elicit an antigenic or immunogenic response. The term "modulating the immune response" refers to the ability of the antigenic preparations of the present invention to stimulate or enhance the immune response, for example as demonstrated by their ability to stimulate the proliferation of lymphocytes in cell culture, (a detailed review can be found in Strube et al. (1989) Vet. Med. Rev., 60, pp. 3-15, Büttner M. (1993) Comp. Immun. Microbiol. Infect. Dis., 16, No. 1, pp. 1-10).

It has now been surprisingly found, that antigenic preparations preparable from keratinophilic fungi or yeast can be used for the prophylaxis and treatment of

allergies, as well as for modulating the immune response, particularly in mammals.

Processes for preparing antigenic material from keratinophilic fungi as well as yeasts have now been developed. The antigenic preparations preparable according to these processes comprise polysaccharides and/or glycopeptides. The antigenic preparations can be used as pharmaceutical compositions as well as vaccines for the treatment of animals and humans, especially for the treatment of allergies and for modulating the immune response. It will be understood that the pharmaceutical compositions of this invention can have immunological as well as pharmacological utility.

The antigenic material of this invention may also be prepared from material derived from keratinophilic fungi or yeasts, for example from the fungal or yeast cell walls.

For the preparation of the antigenic preparations of the present invention, three different processes have been developed. According to these processes three different antigenic fractions (ASMP, ANMP or AEMP), in the following commonly referred to as "fractions", can be prepared from keratinophilic fungi as well as yeasts. Antigenic preparations comprising more than one fraction are referred to in the following as "complex preparation" or abbreviated "Complex".

Process 1: The fraction preparable according to this process consists of antigenic soluble material comprising polysaccharide and/or glycopeptides (ASMP).

Briefly this process, which is illustrated in detail in Example 1, comprises the following:

Keratinophilic fungi or yeasts are cultivated on Agar plates, for example as described in EP 0564620. One preferred medium is for example malt extract agar from Oxoid. Other media that will ensure growth of keratinophilic fungi or yeast may be used as well. The resulting fungal biomass is lifted off and treated with an aqueous solution of alkali. Preferred aqueous alkaline solutions are NaOH or KOH at preferred concentrations of 0.1-5%(w/v). Alkaline treatment is preferably at 20 -150 C for up to 30h. Following the processing under aqueous alkaline conditions, the solid and liquid phases of the preparation are separated, for example by centrifugation, filtration or sedimentation. Preferably the separation is achieved by centrifugation, which ensures good separation of the fungal cell debris, for example at forces of about 3500g. The treatment under aqueous alkaline conditions, as well as the separation step, may be repeated several times.

After the alkaline treatment, the resulting supernatant is treated under acidic aqueous conditions, e.g. 0.2-1.5M organic acid or 0.05-1M mineral acid. For example HCl or acetic acid can be used, preferably at pH values between pH 2.5 and pH 4.5. Preferably the treatment under aqueous acidic conditions is for 2 to 4 hours at temperatures of 4 to 8 C, whereafter separation of the solid and liquid layers takes place. The treatment under aqueous acidic conditions, as well as the separation step, may be repeated several times, preferably under conditions as above indicated. Then,

the supernatant from the separation step is subject to a precipitation step. Preferably the precipitation is performed by adding a suitable organic solvent, e.g. an alcohol such as a lower alkanol to the supernatant, for example methanol or ethanol. A ratio of one volume supernatant to 2-5 volumes of alcohol will result in good precipitation of the antigenic material. Other non-alcoholic precipitation procedures known to the person skilled in the art may be used as well, for example ammonium sulphate or other salt precipitation may result in precipitation of the antigenic material as well. The solid phase is then subject to a further separation step, preferably under conditions as described above. The resulting solid phase is recovered and if desired is dissolved in an aqueous solution, preferably in distilled water, typically 25 to 100 ml are used. Finally the ASMP preparation can be lyophilised and stored for prolonged time periods under dry conditions.

Process 2: The fraction preparable according to this process consists of antigenic nonsoluble material comprising polysaccharide and/or glycopeptides (ANMP). Briefly this process, which is illustrated in detail in Example 2, comprises the following:

Keratinophilic fungi or yeasts are cultivated on Agar plates, for example as described in EP 0564620. A preferred medium is for example malt extract agar from Oxoid. Other media that will ensure growth of keratinophilic fungi or yeast may be used as well. The resulting fungal biomass is lifted off and treated with an aqueous solution of alkali. Preferred aqueous

alkaline solutions are NaOH or KOH at preferred concentrations of 0.1-5%(w/v). Alkaline treatment is preferably at 20-150 C for up to 30h. Following the processing under aqueous alkaline conditions, the solid and liquid phases of the preparation are separated, for example, by centrifugation, filtration or sedimentation. Preferably the separation is achieved by centrifugation, which ensures good separation of the fungal cell debris, for example at forces of about 3500g. The treatment under aqueous alkaline conditions may be repeated several times, as well as the separation step. After alkaline treatment, the solid phase is treated with mineral or organic acids. Preferably 0.2-1.5 M acetic acid or 0.05-1 M HCl are added to the solid phase for 0.5 to 3 hours at temperatures of 70 to 100 C. After acidic treatment the solid phase is washed with an aqueous solution, preferably distilled water. Advantageously the washing is repeated about five times. Finally the solid phase is suspended in distilled water.

Process 3: The fraction preparable according to this process consists of antigenic exogenous material comprising polysaccharide and/or glycopeptides (AEMP). Briefly this process, which is illustrated in detail in Example 3, comprises the following:

Keratinophilic fungi or yeasts are incubated in aqueous solution or cultivated in liquid medium for up to 240 hours (the volume of the solution or culture is here defined as primary volume PV). Distilled water can be used (see example 3. I.) as well as media described in EP 0564620. After incubation or cultivation, the

fungal cells are separated, for example, by centrifugation, filtration or sedimentation, preferably by centrifugation under conditions as described above. The resulting supernatant is then lyophilised and subsequently dissolved in water. Preferably the volume of water equals 0.1 to 0.2 volumes of the primary volume (PV). The resulting solution is then subject to a precipitation step. Preferably the precipitation is performed by adding a suitable organic solvent, e.g. an alcohol such as a lower alkanol to the supernatant, for example methanol or ethanol. A ratio of one volume supernatant to 2-5 volumes of alcohol will result in good precipitation of the antigenic material. Other non-alcoholic precipitation procedures known to the person skilled in the art may be used as well, for example ammonium sulphate or other salt precipitation may result in precipitation of the antigenic material as well. The resulting precipitate is recovered and if desired is dissolved in an aqueous solvent, preferably in distilled water. Preferably 0.5 to 50mg of the precipitate are dissolved in 1ml of aqueous solvent. Finally the AEMP solution can be lyophilised and stored for prolonged time periods under dry conditions, preferably at 2 to 10 C.

Preferred fungal genera from which the above defined Fractions are preparable are the genera Trichophyton, Microsporum or Candida.

Preferred species are:

- Trichophyton equinum,
- Trichophyton mentagrophytes,
- Trichophyton sarkisovii,

- Trichophyton verrucosum,
- Microsporum canis,
- Microsporum gypseum, or
- Candida albicans.

Preferred strains of the above referenced species are:

- Trichophyton equinum DSM No. 7276,
- Trichophyton mentagrophytes DSM No. 7279,
- Trichophyton sarkisovii DSM No. 7278,
- Trichophyton verrucosum, DSM 7277,
- Microsporum canis DSM No. 7281,
- Microsporum canis var. obesum DSM No. 7280,
- Microsporum canis var. distortum DSM No. 7275,
- Microsporum gypseum DSM No. 7274, or
- Candida albicans, DSM No. 9656.

All above referenced strains have been deposited by the applicant at the DSM ("Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH", Mascheroder Weg 1B, D-38124 Braunschweig, Germany) under the provisions of the Budapest Treaty on the deposition of micro-organisms. All strains except Candida albicans DSM No. 9656 have been previously described in the USSR Patent Application No. 5006861 filed 21.10.1991, and corresponding applications i.e. the published Patent Application EP 0564620, filed on 17.10.1992.

Depending on the species the fractions can be obtained from, they are referred to according to the following.

Fractions derived from:

(i) Trichophyton equinum, are referred to as ASMP-TE, ANMP-TE, or AEMP-TE,

(ii) Trichophyton mentagrophytes, are referred to as ASMP-TM, ANMP-TM, or AEMP-TM,

(iii) Trichophyton sarkisovii, are referred to as ASMP-TS, ANMP-TS, or AEMP-TS,

(iv) Trichophyton verrucosum, are referred to as ASMP-TV, ANMP-TV, or AEMP-TV,

(v) -Microsporum canis, are referred to as ASMP-MC, ANMP-MC, or AEMP-MC,

(vi) Microsporum gypseum, are referred to as ASMP-MG, ANMP-MG, or AEMP-MG, or

(vii) Candida albicans, are referred to as ASMP-CA, ANMP-CA, or AEMP-CA.

Where information with regard to the specific strain is given, the species abbreviation is followed by the digits of the specific DSM deposit, for example - AEMP-CA9656 refers to the AEMP fraction preparable from Candida albicans strain DSM No. 9656.

The Fractions preparable as defined in any one of the above described processes (1 to 3) comprise at least one single antigen preparable from at least one of the above referenced fungi. The antigenic preparations of the present invention comprise at least one of the above defined fractions or combinations thereof.

The antigenic preparations (ASMP and AEMP) as described in Examples 1 and 3:

1) comprise monosaccharides, amino acids and nucleotides, which are bound to a large extend in polymeric structures and to a smaller portion are free monomers.

2) mainly consist of the monosaccharide units: mannose galactose, glucose and xylose and others in different relative amount.

3) contain a mixture of polymeric structures formed by a significant amount of these monosaccharides. A significant part of these polymeric structures show molecular weights greater than 20 000 kD.

4) contain low amounts of free or bound amino acids.

5) contain low amounts of DNA molecules shown to be sensitive to digestion with DNase I.

NMR spectroscopy of the antigenic preparations ASMP and AEMP resulted in the NMR spectrograms presented in Figures 1 to 4.

The chemical shifts and signal multiplicities (summarized in Table 12) are in agreement with literature data for carbohydrates and amino acids.

For AEMP and ASMP fractions, e.g. MG 7274, TM 7279 and CA 9656, the carbohydrate signals cover a range from 3.2 - 5.5 ppm, the amino acid signals a region from 0.75 - 3.45 (without α-protons).

ASMP also shows typical signals for acetate-CH_3 1.92ppm.

The AEMP fractions show also typical signals for disacharides and amino acids. E.g. the TM 7279 spectrum shows signals for aromatic amino acids like Phenylanalanine, Tyrosine and Tryptophane in the region 7.15 - 7.9 ppm.

Concerning single fractions of ASMP or AEMP, concentrations of 0.1 to 50mg/ml are preferred. Concerning single Fractions of ANMP, concentrations of 0.1 to 5%(v/v) are preferred.

Preferred embodiments of the antigenic preparations of the present invention comprise for example the following combinations of Fractions (Complexes):

Complex 1 comprises ASMP-TM, and ASMP-MG, and ASMP-CA. Preferably the concentration of each fraction is 0.1 to 50mg/ml. A highly preferred embodiment according to Complex 1 is a combination of ASMP-TM7279, ASMP-MG7274, and ASMP-CA9656.

Complex 1.1 comprises ASMP-MG and ASMP-CA. Preferably the concentration of each fraction is 0.1 to 50mg/ml. A highly preferred embodiment according to Complex 1.1 is a combination of ASMP-MG7274 and ASMP-CA9656.

Complex 2 comprises ANMP-TM, and ANMP-MG, and ANMP-CA. Preferably the concentration of each fraction is 0.1 to 5%(v/v). A highly preferred embodiment according to Complex 2 is a combination of ANMP-TM7279, ANMP-MG7274, and ANMP-CA9656.

Complex 3, comprises AEMP-TM, and AEMP-MG, and AEMP-CA. Preferably the concentration of each fraction is 0.1 to 50mg/ml. A highly preferred embodiment according to Complex 3 is a combination of AEMP-TM7279, AEMP-MG7274, and AEMP-CA9656.

Complex 4 comprises ANMP and AEMP. The following combinations of fractions are preferred: (1) ANMP-CA and AEMP-TM or (2) ANMP-MG, ANMP-TM and AEMP-TM. Preferably the concentration of ANMP is 0.1 to 5%(v/v) and that of AEMP is 0.1 to 50mg/ml. Highly preferred embodiments according to Complex 4 are the following combinations:

4.1 ANMP-CA9656, and AEMP-TM7279;

4.2 ANMP-MG7274, and ANMP-TM7279, and AEMP-TM7279;

Complex 5, comprises ANMP and ASMP. A preferred combination is ANMP-MG, and ANMP-TM, and ASMP-CA. Preferably the concentration of the individual ANMP

fractions is 0.1 to 5%(v/v), and that of individual ASMP fractions is 0.1 to 50mg/ml. Highly preferred is a combination of ANMP-MG7274, and ANMP-TM7279, and ASMP-CA9656.

Further preferred antigenic complexes according to the present invention comprise for example: ASMP and AEMP-or ASMP and AEMP and ANMP at concentrations for ASMP and AEMP of 0.1-50mg/ml and for ANMP at concentrations of 0.1 to 5%(v/v).

The antigenic preparations of the present invention can be applied together with suitable physiologically acceptable carriers that do not cause adverse physiological side effects, and include buffers, solutions or adjuvants, for example salt solutions, Lactate solutions or Ringer Solution. Preferred carriers are for example: Carrier A: aqueous solution comprising 0.85%(w/v) NaCl; Carrier B: aqueous solution comprising 5%(w/v) Glucose, 0.3%(w/v) meat extract "lab-lemco" (Oxoid), and 0.1%(w/v) yeast extract (Oxoid); Carrier C: Medium RPMI 1640 (purchased from Serva, catalogue no 12-702).

The antigenic preparations of the present invention can be applied *per se* or as solutions for injection, creams, sprays, aerosols, tablets and in other application forms known to the person skilled in the art. The antigenic preparations of the present invention may further provide highly efficient vaccines.

The antigenic preparations of the present invention are able to stimulate the proliferation of cells of the immune system and thereby are able to modulate the immune response. The antigenic preparations of the

present invention are further able to inhibit the proliferation of human keratinocytes.

The antigenic preparations of the present invention may confer a high degree of resistance against allergic reactions, particularly of epithelial tissues, more particularly of the skin. They are of interest for preventing and curing allergy, and in our hands have not shown adverse side effects as demonstrated *in vivo* in laboratory animals (i.e. guinea pigs and white mice) and horses (i.e. cross-breed and Icelandic horses).

In particular, acute allergic dermatitis and skin lesions may be effectively cured without side effects by administering the antigenic preparation of the present invention, i.e. by vaccination. After intra muscular injection(s) of the antigenic preparations of the present invention, the symptoms of allergic inflammation of the skin, itch and the sensitivity of the skin of individuals afflicted with allergic dermatitis may be abolished. Complete recovery from all allergic symptoms has been achieved within 2 to 8 weeks after the final injection and the allergen induced sensitivity of the skin to irritants was abolished. Further, within 1 to 6 weeks after the final injection itch may be abolished.

In a preferred embodiment, the antigenic preparations of the present invention provide a protection and cure for the so called Summer Eczema of horses, especially of Icelandic horses. After 1 to 3 intra muscular or intra dermal injection(s) of the antigenic preparations of the present invention, horses afflicted with Summer Eczema may be cured of or protected against Summer Eczema, preferred are complexes 1 and 1.1.

In a further preferred embodiment, the antigenic preparations of the present invention provide a protection and cure against alopecia in mammals. After 1 to 3 intra muscular or intra dermal injection(s) of the antigenic preparations of the present invention, mammals afflicted with alopecia may be cured of or protected against alopecia, preferred are Complexes 1 or 1.1.

In another preferred embodiment, the antigenic preparations of the present invention improve the hair condition and seasonal coat change of mammals. After 1 to 3 intramuscular or intradermal injections, coat condition may be significantly improved and in individuals afflicted with incomplete coat change complete change to the regular season coat may result, preferred are Complexes 1 or 1.1.

In another preferred embodiment, the antigenic preparations of the present invention provide a protection and cure against eczema. After 1 to 3 intra dermal or intramuscular injection(s) of or after topical treatment with the antigenic preparations of the present invention, mammals, i.e. humans, afflicted with eczema, may be cured of or protected against eczema, preferred are fractions ASMP-MG, ASMP-CA and ASMP-TM, i.e. ASMP-MG7274, ASMP-CA9656 and ASMP-TM7279 or complexes 1 and 1.1.

In a further preferred embodiment, the antigenic preparations of the present invention provide a protection and cure against neurodermitis. After topical treatment with the antigenic preparations of the present invention, mammals, i.e. humans, afflicted with neurodermitis, may be cured of or protected against neurodermitis, preferred are fractions ASMP-MG, ASMP-CA

and ASMP-TM, i.e. ASMP-MG7274, ASMP-CA9656 and ASMP-TM7279 or complexes 1 and 1.1.

The antigenic preparations of the present invention may be used to treat a variety of indications such as those described in "Klinische Immunologie", Peter, H.H. (editor), publ. 1991 by Urban & Schwarzenberg, Munich, Germany, for example:

1. allergic airway diseases
    - 1.1. allergic rhinitis and conjunctivitis
        - 1.1.1. seasonal rhino-conjunctivitis
        - 1.1.2. perennial rhinitis
    - 1.2. asthma bronchiale
    - 1.3. status asthmaticus
    - 1.4. asthma of children
        - 1.4.1. obstructive lung disease after infectious bronchiolitis
        - 1.4.2. mild episodic or mild perennial asthma bronchiale
        - 1.4.3. strong perennial asthma bronchiale

2. allergic broncho pulmonary aspergillosis

3. food allergies
    - 3.1. IgE-mediated food allergy
        - 3.1.1. IgE-mediated food allergy of infants
        - 3.1.2. IgE-mediated food allgery of juveniles and adults
    - 3.2. IgG- and T-cell-mediated food allergies
    - 3.3. Intolerance to cow's milk
    - 3.4. Heiner-syndrome
    - 3.5. eosinophilic gastroenteropathy

3.6. coeliac disease

4. Insect bite/sting allergy

5. urticaria in all its forms
   - 5.1. contact urticaria
   - 5.2. urticaria concomitant with allergic reactions
   - 5.3. urticaria concomitant with intolerance to additives and inhibitors of prostaglandin synthesis (pseudo-allergy)
   - 5.4. physical urticaria
     - 5.4.1. dermatographia (urticaria factitia)
     - 5.4.2. cholinergic and adrenergic urticaria
     - 5.4.3. cold-induced urticaria
     - 5.4.4. light urticaria
     - 5.4.5. pressure urticaria
     - 5.4.6. other rare forms of physical urticaria
   - 5.5. urticarial vasculitis
   - 5.6. mastocytosis and urticaria pigmentosa
   - 5.7. urticaria concomitant with infectious diseases
   - 5.8. urticaria concomitant with immunothyroiditis
   - 5.9. urticaria and amyloidosis

6. angioedema
   - 6.1. hereditary angloneuroticoedema (HANE)
   - 6.2. acquired angioneuroticoedema

7. atopic dermatitis, atopic eczema

8. drug related allergy

Table 1: Properties and characteristics of Candida albicans DSM No. 9656

Properties and characteristics of the strain	DSM No. 9656	Epidemic strain No. 008
Description of culture	10-day colony on Saboraud agar is cream smooth, pasty, glistening, and elevated, with a central depression, the margin of the colony is regular, with a diameter of 18-22 mm	10-day colony on Saboraud agar is cream smooth, pasty, and glistening, with folded segments, the margin of the colony is irregular, with a diameter of 15-18 mm
Morphological characteristics	spherical oval blasto-spores measure 3.5-5x5-8 µm, pseudo hyphae are 5-8 µm wide, hyphae are 2-3 µm wide. Chlamydospores on rice agar measure 13-16 µm in diameter	spherical oval blasto-spores measure 3.5-5x5-8 µm, pseudo hyphae are 5-8 µm wide, hyphae are 2-3 µm wide. Chlamydospores on rice agar measure 13-16 µm in diameter
Pathogenic characteristics	30 days after intra peritoneal injection of 10-100 million fungal cells into white mice, 80% of the animals carried abdominal granulomas, no lethal effects are observed	30 days after intra peritoneal injection of 10-100 million fungal cells into white mice, 80% of the animals carried abdominal granulomas, 40 % of the animals died

The present invention further relates to Candida albicans strain DSM No. 9656, which was obtained by directed selection based on stabilisation of cultural-morphological characteristics and attenuation of

epidemic strain No. 008, which was isolated from a man in 1990.

The biological properties of Candida albicans strain DSM No. 9656 are described in Table 1.

Strain Candida albicans DSM No. 9656 further differs from the epidemic strain in its population stability, and morphological characteristics under long term passaging through nutrient media and lower virulence. Following the teachings for the preparation of antigenic preparations of the present invention, highly effective and safe antigenic preparations, according to the present invention, can be prepared from this strain.

One skilled in the art will readily appreciate that the present invention is well adapted to carry out the objects and attain the ends and advantages mentioned as well as those inherent therein. The compounds, procedures and techniques described herein are presently representative of preferred embodiments, are intended to be exemplary, and are not intended as limitations on the scope.

Having now generally described the present invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention.

Examples

For all examples the centrifugation was performed at forces between 3000g to 3500g for about 30-50 min. The media were purchased from Oxoid (Unipath GmbH, Am Lippeglacis 6-8, 46483 Wesel, Germany) or Serva (Serva

Feinbiochemica GmbH & Co. KG, Carl-Benz-Str. 7, 69115 Heidelberg, Germany). If not indicated otherwise, the fungi were cultivated as described in the Oxoid catalogue "5. aktualisierte deutsche Ausgabe" or in EP 0564 620. Fungus strains used for the preparation of the antigenic preparations of the present invention were obtained by selection and attenuation of fungus strains as described in N.V. Mazkevitch, 1981, "Spontannaja ismentchivost i kariologia nesovershennich gibov", published by Isdatelstwo Nauka, Moscow; and Ivanova, L.G., 1992, "Sistematika, morphologitcheskaja charakteristika, biologitcheskii svojstva vosbuditelej dermatophitosov, obshih dlja givotnih i tcheloveka", Moscow, Library of the University of Moscow. Basic culturing techniques for mammalian cell cultures can be readily found in Doyle, Griffiths, and Newell (Eds.), Cell & Tissue Culture: Laboratory Procedures, John Wiley & Sons (1995). For the keratinocyte assays HaCaT cells were used (Boukamp et al. (1988), J. Cell Biol., 106, pp. 761-771, and Ryle et al. (1989), Differentiation, 40, pp. 42-54) isolated keratinocytes or other keratinocyte cell lines can be used as well. Horse lymphocytes were isolated and cultivated as described in Friemel, H., "Immunologische Arbeitsmethoden", published by VEB Gustav Fischer Verlag, Jena, 1984; or Paul, E., "Fundamental Immunology", published by Raven Press, New York, 1984. Radio assays were essentially performed as described in Boehncke et al., 1994, Scand. J. Immunol. 39, pp. 327-332 and references cited therein. NaOH, KOH, HCl and acetic acid were prepared as aqueous solutions. If not indicated otherwise, the term soluble refers to the solubility in aqueous solution. Physiologically

acceptable carriers used in the experiments described below are in example: Carrier A: aqueous solution comprising 0.85%(w/v) NaCl; Carrier B: aqueous solution comprising 5%(w/v) Glucose, 0.3%(w/v) meat extract "lab-lemco" (Oxoid), and 0.1%(w/v) yeast extract (Oxoid); Carrier C: Medium RPMI 1640 (Serva).

Example 1

Antigenic soluble material comprising polysaccharide and/or glycopeptides (ASMP) was prepared from:

Trichophyton mentagrophytes (ASMP-TM), Microsporum gypseum (ASMP-MG) or Candida albicans (ASMP-CA), according to the following procedures:

Fungi were cultivated on Agar plates as described in EP 0564620. The fungal biomass was lifted off and for the production of:

I. ASMP-TM:

(i) Trichophyton mentagrophytes biomass was treated with 4.5%(w/v) of NaOH at about 140 C for 1 hour followed by centrifugation for 45 minutes. To the supernatant a 4M solution of acetic acid was added until a final pH of 3.5 was reached. After 2 hours the sediment was separated by centrifugation and 3 volumes of ethanol were added to 1 volume of supernatant. The sediment resulting from the alcoholic precipitation was sedimented by centrifugation and dissolved in distilled water. Finally the individual ASMP preparations were lyophilised.

(ii) Trichophyton mentagrophytes biomass was treated with 0.2%(w/v) of KOH at about 140°C for 1 hour followed by centrifugation. The supernatant was treated with a 1M solution of HCl at a final pH of 3.5 for 4 hours at 4-10 C. The sediment was then separated by centrifugation and 2 volumes of ethanol were added to 1 volume of supernatant. The sediment resulting from the alcoholic precipitation was sedimented by centrifugation and dissolved in distilled water. Finally the individual ASMP preparations were lyophilised.

II. ASMP-MG,

(i) Microsporum gypseum biomass was treated with 0.2%(w/v) of NaOH at about 140 C for 2 hours followed by centrifugation. The sediment was again treated with 0.2%(w/v) of NaOH at about 140 C for 2 hours followed by centrifugation and the procedure was repeated for a third time. The final supernatant was then treated with a 8M solution of acetic acid at a final pH of 3.5 for 3 hours at 18-20 C. The sediment was then separated by centrifugation, and 3 volumes of ethanol were added to 1 volume of supernatant. The sediment resulting from the alcoholic precipitation was sedimented by centrifugation and dissolved in distilled water. Finally the individual ASMP preparations were lyophilised.

(ii) Microsporum gypseum biomass was treated either with 3%(w/v) of KOH at about 75 C for 6h followed by centrifugation. The sediment was again treated with 3%(w/v) of NaOH at about 75 C for 6h followed by centrifugation. The final supernatant was then treated

with a 0.5M solution of HCl at a final pH of 3.5 for 4 hours at 4-10 C. The sediment was then separated by centrifugation, and 3 volumes of methanol were added to 1 volume of supernatant. The sediment resulting from the alcoholic precipitation was sedimented by centrifugation and dissolved in distilled water. Finally the individual ASMP-preparations were lyophilised.

III. ASMP-CA:

(i) Candida albicans biomass was treated with 3.0%(w/v) of NaOH at about 75 C for 6h followed by centrifugation. The sediment was again treated with 3.0%(w/v) of NaOH at about 75 C for 6h followed by centrifugation. The final supernatant was then treated with a 12M solution of acetic acid at a final pH of 3.5 for 2 hours at 4-10 C. The sediment was then separated by centrifugation, and 2 volumes of methanol were added to 1 volume of supernatant. The sediment resulting from the alcoholic precipitation was sedimented by centrifugation and dissolved in distilled water. Finally the individual ASMP preparations were lyophilised.

(ii) Candida albicans biomass was treated with 4.5%(w/v) of KOH at about 35 C for 3h followed by centrifugation. The sediment was again treated with 4.5%(w/v) of NaOH at about 35 C for 3h followed by centrifugation, and the procedure was repeated for a third time. The final supernatant was then treated with a 0.25M solution of HCl at a final pH of 3.5 for 4 hours 18-20 C. The sediment was then separated by centrifugation, and 2

volumes of ethanol were added to 1 volume of supernatant. The sediment resulting from the alcoholic precipitation was sedimented by centrifugation and dissolved in distilled water. Finally the individual ASMP preparations were lyophilised.

Example 2

Antigenic nonsoluble material comprising polysaccharide and/or glycopeptides (ANMP) was prepared from: Trichophyton mentagrophytes (ANMP-TM), Microsporum gypseum (ANMP-MG) or Candida albicans (ANMP-CA) according to the following procedures:

Fungi were cultivated on Agar plates as described in EP 0564620. The fungal biomass was lifted off and for the production of:

I. ANMP-TM:

(i) Trichophyton mentagrophytes biomass was treated with 0.2%(w/v) NaOH at about 35 C for 24h followed by centrifugation. The sediment was treated with 0.3M acetic acid for about 3 hours at about 60 C and washed five times with distilled water. Each washing step was followed by centrifugation. The final sediment was resuspended in an aqueous solution of 0.85%(w/v) NaCl (Carrier A) to a final concentration of 0.5%(v/v) of ANMP-TM. The ANMP-TM preparation was stored as suspension at 2-10 C.

(ii) Trichophyton mentagrophytes biomass was treated with 0.2%(w/v) KOH at about 35 C for 24h followed by

centrifugation. The sediment was treated with 0.1 M HCl for 30 minutes at 70 C and washed five times with distilled water. Each washing step was followed by centrifugation. The final sediment was resuspended in RPMI 1640 (Carrier C) to a final concentration of 1.5%(v/v) of ANMP-TM. The ANMP-TM preparation was stored as suspension at 2-10 C.

II. ANMP-MG:

(i) Microsporum gypseum biomass was treated with 3%(w/v) NaOH at about 75 C for 6h followed by centrifugation. The sediment was treated again with 3%(w/v) NaOH at about 75 C for 6h followed by centrifugation. The resulting sediment was treated with 0.7M acetic acid for about 4 hours at 60 C and washed five times with distilled water. Each washing step was followed by centrifugation. The final sediment was resuspended in an aqueous solution comprising 5%(w/v) glucose, 0.1%(w/v) yeast extract from Oxoid, and 0.3%(w/v) meat extract "lab lemco" from Oxoid (Carrier B) to a final concentration of 2.5%(v/v) of ANMP-MG. The ANMP-MG preparation was stored as suspension at 2-10 C.

(ii) Microsporum gypseum biomass was treated with 3%(w/v) KOH at about 35°C for 3h followed by centrifugation. The sediment was treated again with 3%(w/v) KOH at about 35°C for 3h followed by centrifugation, and the procedure was repeated a third time. The resulting sediment was treated with 0.5M HCl for 30 minutes at 80 C and washed five times with distilled water. Each washing step was followed by

centrifugation. The final sediment was resuspended in RPMI 1640 (Carrier C) to a final concentration of 2.0%(v/v) of ANMP-MG. The ANMP-MG preparation was stored as suspension at 2-10 C.

III. ANMP-CA:

(i) Candida albicans biomass was treated with 4.5%(w/v) NaOH at about 140 C for 2 hours followed by centrifugation. The sediment was treated again with 4.5%(w/v) NaOH at about 140 C for 2 hours followed by centrifugation, and the procedure was repeated a third time. The resulting sediment was treated with 1M acetic acid for 1 hour at 60 C and washed five times with distilled water. Each washing step was followed by centrifugation. The final sediment was resuspended in an aqueous solution of 0.85%(w/v) NaCl (Carrier A) to a final concentration of 1.5%(v/v) of ANMP-CA. The ANMP-CA preparation was stored as suspension at 2-10 C.

(ii) The Candida albicans biomass was treated with 4.5%(w/v) KOH at about 140 C for 2 hours followed by centrifugation. The sediment was treated again with 4.5%(w/v) NaOH at about 140 C for 2 hours, and the resulting sediment was treated with 0.1 M HCl for 30 minutes at 100 C and washed five times with distilled water. Each washing step was followed by centrifugation. The final sediment was resuspended in RPMI 1640 (Carrier C) to a final concentration of 2.5%(v/v) of ANMP-CA. The ANMP-CA preparation was stored as suspension at 2-10 C.

Example 3

Antigenic exogenous material comprising polysaccharide and/or glycopeptides (AEMP), was prepared from liquid cultures of: Trichophyton mentagrophytes (AEMP-TM), Microsporum gypseum (AEMP-MG) or Candida albicans (AEMP-CA). The liquid cultures were cultivated under conditions essentially as described in EP 0564620. The individual AEMP preparations were obtained according to the following procedures.

I. AEMP-TM: Trichophyton mentagrophytes was incubated for 240h at 26 C in 1000ml distilled water. Then, the culture, containing about 1.2×10^8 cells per ml, was centrifuged. The supernatant was lyophilised and dissolved in 100ml of distilled water, 3 volumes of methanol were added and the precipitate was dissolved in aqueous solution. The supernatant was lyophilised resulting in AEMP-TM.

II. AEMP-MG: Microsporum gypseum was cultivated for 50 h at 28 C in 200ml of Carrier C (RPMI 1640 medium from Serva). The culture, containing about 3×10^7 cells per ml, was centrifuged. The supernatant was lyophilised and dissolved in 20ml of distilled water, 2 volumes of methanol were added and the precipitate was dissolved in aqueous solution. The supernatant was lyophilised resulting in AEMP-TM.

III. AEMP-CA: Candida albicans was cultivated for 30h in 800ml of Carrier B (1%(w/v) meat extract lab-lemco from

Oxoid, 0.1%(w/v) yeast extract from Oxoid and 5%(w/v) dextrose) at 37 C. The culture, containing about 10^8 cells per ml, was centrifuged. The supernatant was lyophilised and dissolved in a small amount of distilled water, 2 volumes of methanol were added and the precipitate was dissolved in aqueous solution. The supernatant was lyophilised resulting in AEMP-TM.

Example 4

The influence of different antigenic preparations on the growth of keratinocyte cell cultures (HaCaT cell cultures) was determined.

I. Antigenic fractions ASMP-TM, ANMP-TM, and AEMP-TM prepared from Trichophyton mentagrophytes DSM No. 7279, ASMP-MG, ANMP-MG, and AEMP-MG prepared from Microsporum gypseum DSM No. 7274, and ASMP-CA, ANMP-CA, and AEMP-CA prepared from Candida albicans DSM No. 9656 were used in different concentrations. The ANMP fractions as prepared according to Example 2 were lyophilised and resuspended in PBS (Phosphate Buffered Saline with a phosphate concentration of 6.7mM at physiological pH of about 7.2; purchased from Serva, Catalogue No 17-516).

For cultivation 12 well tissue culture plates from Falcon (flat bottom, surface area 9.6cm^2) were used. To each well, 0.15 ml keratinocyte cell suspension (HaCaT cells) of about 1 million cells per ml nutrient medium (RPMI 1640 supplemented with 10%(w/v) foetal calf serum), 2 ml of nutrient medium, and 0.02 - 0.1 ml antigenic fraction dissolved in PBS were added. To control wells no antigenic fraction material was added.

Cultivation was performed in an incubator with 5% (v/v) CO_2 at a temperature of 37 C for about 48 hours until a confluent cell mono layer had developed in the control wells.

Inhibition of cell growth was determined by comparing the area size of cell sheets treated with the antigenic fractions compared to controls not treated with antigenic fractions (control = 100%). The results are shown in Tables 2 and 3.

Inhibition of cell growth was observed at an ASMP-MG concentration of 0.1mg/ml, an ASMP-TM concentration of 0.3mg/ml, and an ASMP-CA concentration of 1mg/ml. For ANMP (MG, TM, and CA) inhibition was observed at a concentration of 1 mg/ml. For AEMP-MG, inhibition of cell growth was observed at a concentration of 0.3mg/ml, and for AEMP-TM and AEMP-CA at a concentration of 1mg/ml.

Example 5

The influence of different antigenic fractions on the cell proliferation of horse lymphocytes was determined.

Antigenic fractions ASMP and AEMP of the fungal strains T. mentagrophytes DSM No. 7279, M. gypseum DSM No. 7274; and C. albicans DSM No. 9656 were used. A suspension of about 40 000 lymphocytes (from Icelandic horses) per ml of nutrient medium was prepared. Nutrient medium RPMI 1640 was supplemented with 10% (w/v) foetal calf serum. Cultivation of the lymphocytes was performed in 96 well U-bottom tissue culture plates (Falcon No 3077). 200 µl of cell suspension was distributed to each

well and 20 µl of antigenic fraction dissolved in PBS was added. Controls were performed without addition of antigenic fraction material.

The tissue culture plates were incubated at 37 C with 5%(v/v) CO_2 for 72 hours. Then the nutrient medium was changed and a H^3-Thymidine-containing solution (1 µl per well) was added. A second cultivation step for 12 hours was performed, the culture was washed with PBS. Cell proliferation was determined by radio assay techniques as described in Boehncke et al., 1994, Scand. J. Immunol. 39, pp. 327-332. Measurement of the cell proliferation was performed by comparing the test cultures with the controls not exposed to antigenic fraction material. The control values were defined as 100%. The result is shown in Table 4. The individual antigenic fractions either had an inhibiting or stimulating effect on lymphocyte cell proliferation.

Example 6

This example illustrates typical complex preparations. The complexes (1 to 5) described in this example have been prepared from Trichophyton mentagrophytes DSM No. 7279, Microsporum gypseum DSM No. 7274 or Candida albicans DSM No. 9656.

I. Complex 1 comprises ASMP-TM, ASMP-MG, and ASMP-CA in a suitable carrier, in example

Complex 1	Concentration [mg/ml] A	B	C
ASMP-TM7279	5	10	30
ASMP-MG7274	5	10	30
ASMP-CA9656	5	10	30
	in carrier A or B or C	in carrier A or B or C	in carrier A or B or C

Complex 1.1 comprises ASMP-MG, and ASMP-CA in a suitable carrier, in example

Complex 1	Concentration [mg/ml] A	B	C
ASMP-MG7274	5	10	30
ASMP-CA9656	5	10	30
	in carrier A or B or C	in carrier A or B or C	in carrier A or B or C

II. Complex 2 comprises ANMP-TM, ANMP-MG, and ANMP-CA in a suitable carrier, in example

Complex 2	Concentration [%(v/v)] A	B	C	D
ANMP-TM7279	0.5	1.0	1.5	2.5
ANMP-MG7274	0.5	1.0	1.5	2.5
ANMP-CA9656	0.5	1.0	1.5	2.5
	suspension in carrier A or B or C	suspension in carrier A or B or C	suspension in carrier A or B or C	suspension in carrier A or B or C

III. Complex 3 comprises AEMP-TM, AEMP-MG, and AEMP-CA in a suitable carrier, in example

	Concentration [mg/ml]		
Complex 3	A	B	C
AEMP-TM7279	5	10	30
AEMP-MG7274	5	10	30
AEMP-CA9656	5	10	30
	in carrier A or B or C	in carrier A or B or C	in carrier A or B or C

IV. Complex 4 comprises ANMP and AEMP in a suitable carrier, in example

(i) Complex

4.1 ANMP-CA9656 2.5%(v/v)
AEMP-TM7279 7.1 mg/ml
in carrier A or B or C

(ii)	Concentration	
Complex 4.2	A	B
ANMP-MG7274	2.5%(v/v)	3.0%(v/v)
ANMP-TM7279	2.5%(v/v)	3.0%(v/v)
AEMP-TM7279	10.5mg/ml	18.5mg/ml
	in carrier A or B or C	in carrier A or B or C

V. Complex 5 comprises ASMP and ANMP in a suitable carrier, in example

Complex 5	Concentration A	Concentration B
ANMP-MG7274	1.75% (v/v)	3% (v/v)
ANMP-TM7279	1.75% (v/v)	3% (v/v)
ASMP-CA9656	15.6mg/ml in carrier A or B or C	15.6mg/ml in carrier A or B or C

Example 7

The safety of different antigenic preparations was tested in vaccination experiments in animal model systems (white mice, guinea pigs, and horses).

Antigenic fractions were prepared as described in Examples 1 to 3 and 6 from Trichophyton mentagrophytes DSM No. 7279, Microsporum gypseum DSM No. 7274, or Candida albicans DSM No. 9656.

The following clinical observations concerning the condition of the vaccinated animals were made daily up to five days after each vaccination:

1. Common Condition
   - appetite
   - influence on locomotion

2. Local reaction
   - oedema and inflammation at the injection site
   - changes of the temperature at the injection site
   - development of pain at the injection site

- necessity to treat the injection site with medicaments

I. Antigenic preparations were injected one or two times with an interval of 10 days intra abdominally into white mice and intra abdominally and sub cutaneously into guinea pigs. The antigenic preparations, their concentrations and the results are shown in Tables 5 and 6 (A and B). The subcutaneous or intra abdominal injection of the fungal antigens as single or complex preparations mostly had no adverse effect on the general condition of the animals and a local reaction at the injection site was not observed.

II. Complex preparations of the fungal antigens as described in Example 6 (Complexes 4.1, 4.2, and 5) were each once injected intra muscular into the same horse at different locations (left and right side of the neck and in one of the chest muscles. Three different horses were vaccinated: (i) one pregnant mare, (ii) one foal, age : 7-8 months, and (iii) one stallion, age: 6 years. The antigenic preparations, their concentrations and the results are shown in Table 7.

The intra muscular injection of the fungal antigens as complex preparation had no influence on the general condition of the horses and a local reaction at the injection site was not observed. These studies demonstrate the excellent safety of the antigenic preparations of the present invention.

Example 8

The influence of different antigenic preparations on the condition of skin and hairy coat was studied in white mice.

The antigenic preparations were prepared as described in Examples 1 to 3 and 6 from Trichophyton mentagrophytes DSM No. 7279, Microsporum gypseum DSM No. 7274, or Candida albicans DSM No. 9656.

The antigenic preparations were injected two times in an interval of 10 days intra abdominally into white mice. Observation of the condition of skin and hairy coat continued for five days. The antigenic preparations, their concentrations and the results are shown in Table 8. Injections of the antigenic preparations improved the condition of skin and hairy coat of white mice, as compared to control animal afflicted with dermatitis.

Example 9

The efficacy of three different antigenic preparations was studied by vaccination of Icelandic horses afflicted with Summer Eczema in a placebo controlled trial.

The antigenic preparations were prepared as described in Examples 1 to 3 and 6 from T. mentagrophytes DSM No. 7279, M. gypseum DSM No. 7274 and C. albicans DSM No. 9656. A volume of 1 ml of Carrier A containing the individual antigenic preparations was injected three times intra muscularly. The interval between each injection was five days. Injections were administered alternately in the right and left side of the chest muscle. The antigenic preparations, their

concentrations and the results are shown in Tables 9 and 10.

Administration of an antigenic preparation comprising ASMP-MG7274, ASMP-TM7279, and ASMP-CA9656 resulted in the complete cure of all vaccinated horses (3) four weeks after the third injection. The horses of the control group (injection of Carrier A without antigens) did not show any signs of recovery.

Example 10

The safety of three different antigenic preparations was studied by vaccination of Icelandic horses afflicted with Summer Eczema in a placebo controlled trial.

The antigenic preparations were prepared as described in Examples 1 to 3 and 6 from T. mentagrophytes DSM No. 7279, M. gypseum DSM No. 7274 and C. albicans DSM No. 9656. A volume of 1 ml of Carrier A containing the individual antigenic preparations was injected three times intra muscularly. The interval between each injection was five days. Injections were administered alternately in the right and left side of the chest muscles. Animals were observed for side effects during a time span of three days after each injection. The antigenic preparations, their concentrations and the results are shown in Table 11. General side effects like fever or loss of appetite were not observed. Only one of the antigenic preparations induced swelling at the injection-side. This minor side effect was observed in only one horse. No signs of pain were observed.

Example 11

The antiallergic efficacy of single fractions ASMP-TM7279, ASMP-MG7274 and ASMP-CA9656 as well as of complex 1 comprising ASMP-TM7279, ASMP-MG7274 and ASMP-CA9656 has been studied in a laboratory animal model.

Single fractions have been prepared according to example 1. Complex 1 was prepared according to examples 1 and 6.

CF-1 mice have been sensitzed following the modell and instructions of the Mouse Ear Swelling Test (Gad SC, Dimm BK, Dobbs DW, Reilly C, Walsh RD: Development and Validation of an Alternative Dermal Sensitization Test: The Mouse Ear Swelling Test (MEST). Toxicology and Applied Pharmacology 84, 93-114, 1986. This is a well known, validatet and OECD accepted test to examine allergic substances. To prove the efficacy of the complex or its single fractions for its anti allergic potency in a laboratory animal ear swelling which is caused by the allergene should be prevented. A placebo controled blind study with mice and two different allergens had been conducted:

The MEST was performed with CF-1 mice, which are most sensitive for allergenes. 6-10 week old CF-1 mice, have been prepared by shaving the abdominal skin, injecting 0,05ml of Freund`s Adjuvans and applying 100µl of the allergene 1-chloro-2,4-dinitrocholorbenzene (DNCB) in one trial and mite allergene in another trial topically to the shaved abdominal skin from day 0 to 4. Seven days later 20µl of the allergene had been applied topically to the test ear, the dissolving solution to the control ear. 24 and 48 h later the ear thickness have been measured. The same procedure has been carried

out with the control group, which has been treated with placebo instead of the complex respectively the fractions of the complex.

Administration of single fractions ASMP-TM7279, ASMP-MG7274 and ASMP-CA9656 as well as of complex 1 comprising ASMP-TM7279, ASMP-MG7274 and ASMP-CA9656 resulted in 90% reduced ear swelling after sensitization with mite allergene and 87,5% reduced ear swelling after sensitization with DNCB 48h following rechallenge in comparison to the control groups.

Example 12

The efficacy of a complex preparaton, comprising antigenic preparations ASMP-MG7274 and ASMP-CA9656, prepared as described in Example 1, was demonstrated by vaccination of an Icelandic horse afflicted with Summer Eczema.

Intradermal injections of a volume of 0.4ml of Carrier A containing 0.2mg of MG and 0.2mg of CA for three times, with an interval of five days between each injection, resulted in the cure of the vaccinated horse three weeks after the final injection, as evidenced by significant decrease of the clinical symptoms. No side effects have been observed.

Example 13

The efficacy of an antigenic preparation prepared as described in Example 1 (ASMP) from Microsporum gypseum DSM No. 7274 was demonstrated by vaccination of an 41 year old man suffering from an eczema with inflammation, itching and erosions on the skin between the 4th and 5th toe.

A volume of 0.1ml of Carrier A containing 0.4mg of ASMP-MG7274 was injected intradermally, once only. The skin turned to normal 4 - 5 days after treatment.

Itching already had disappeared 24h after injection. No severe side effects have been observed.

Example 14

The efficacy of an antigenic preparation prepared as described in Example 1 (ASMP) from Candida albicans DSM No. 9656 in the treatment of neurodermitis was demonstrated.

ASMP-CA9656 was mixed into a cream, using "Kamill Hand und Nagelcreme" purchased from Procter & Gamble, to a final concentration of 60mg ASMP-CA9656/ml cream. The preparation was applied topically to a 3 year old girl suffering from neurodermitis with yellow crusts on the skin near both ears. The cream was applied topically to the defect part of skin once per day for 30 days. After this treatment the skin returned to normal. Side effects have not been observed.

Example 15

The efficacy of an antigenic preparation prepared as described in Example 1 (ASMP) from Microsporum gypseum DSM No. 7274 in the treatment of eczema was demonstrated.

ASMP-MG7274 was mixed into a cream, using "Kamill Hand und Nagelcreme" purchased from Procter & Gamble, to a final concentration of 60mg ASMP-CA9656/ml cream. A 30 year old man suffering of an eczema with inflammation, erosions and itching on the ring finger was treated by topical application of the aflicted parts of the skin

once per day for 30 days. This resulted in complete cure after treatment. Itching had disappeared a few days after treatment start. Side effects have not been observed.

Example 16

-The efficacy of antigenic preparations, prepared as described in Example 1 (ASMP) from Microsporum gypseum DSM No. 7274, Trichophyton mentagrophytes DSM No. 7279, and Candida albicans DSM No. 9656, has been tested by vaccination in a 5 year old horse which had not changed the winter coat till June. A volume of 1ml of Carrier A containing 15mg of each antigenic preparation ASMP-MG7274, ASMP-TM7279 and ASMP-CA9656 (final concentration 45mg ASMP/ml) was injected three times with an interval of five days intramuscularly, what resulted in complete change to regular season coat within 15 days. Side effects have not been observed.

Example 17

The efficacy of a complex antigenic preparations prepared as described in Example 1 (ASMP) from Microsporum gypseum DSM No. 7274, Trichophyton mentagrophytes DSM No. 7279, and Candida albicans DSM No. 9656 for the treatment of alopecia is demonstrated.

Two 7 year old horses suffering from alopecia one 3 - 5 and one on 7 - 10 different locations all over the body were treated with a vaccine containing 10mg of each antigenic preparation ASMP-MG7274, ASMP-TM7279 and ASMP-CA9656 in 1 ml of carrier A (final concentration 30mg/ml). The vaccine was injected three times with an interval of five days intramuscularly, what resulted in

complete restitution of the coat of both horses 10 days after the last application. Side effects have not been observed.

Example 18

The efficacy of a complex antigenic preparation prepared as described in Example 1 (ASMP) from Microsporum gypseum DSM No. 7274, Trichophyton mentagrophytes DSM No. 7279, and Candida albicans DSM No. 9656 for the treatment of alopecia in horses is demonstrated.

A 10 year old horse suffering from alopecia on 10 - 12 different locations all over the body was treated with a vaccine containing 15mg of each antigenic preparation ASMP-MG7274, ASMP-TM7279 and ASMP-CA9656 in 1 ml of carrier A (final concentration 45mg/ml). The vaccine was injected three times with an interval of five days intramuscularly, what resulted in complete restitution of the coat 15 days after the last application. Side effects have not been observed.

Example 19

The efficacy of a complex antigenic preparation prepared as described in Example 1 (ASMP) from Microsporum gypseum DSM No. 7274, Trichophyton mentagrophytes DSM No. 7279, and Candida albicans DSM No. 9656 for the treatment of alopecia in dogs is demonstrated.

A 3 year old female dog suffering from alopecia on 2 - 3 different locations all over the body was treated with a vaccine containing 10mg of each antigenic preparation ASMP-MG7274, ASMP-TM7279 and ASMP-CA9656 in

1 ml of carrier A (final concentration 30mg/ml). The vaccine was injected three times with an interval of five days intramuscularly, what resulted in complete restitution of the coat 15 days after the last application. Side effects have not been observed.

Example 20

The efficacy of a complex antigenic preparation prepared as described in Example 1 (ASMP) from Microsporum gypseum DSM No. 7274, Trichophyton mentagrophytes DSM No. 7279, and Candida albicans DSM No. 9656 for the treatment of alopecia in dogs is demonstrated.

Two male dogs, one 5 years and one 8 years old, suffering from alopecia on 2 - 4 different locations all over the body were treated with a vaccine containing 15mg of each antigenic preparation ASMP-MG7274, ASMP-TM7279 and ASMP-CA9656 in 1 ml of carrier A (final concentration 45mg/ml). The vaccine was injected three times with an interval of five days intramuscularly, what resulted in complete restitution of the coat 30 days after the last application. Side effects have not been observed.

Table 2: Influence of the concentration of different antigenic fractions on the growth of keratinocyte cell cultures (HaCat cells in percent, compared to controls not exposed to antigenic fractions (confluent cell mono layer of controls = 100%)

antigenic fraction	concentration of antigenic fraction [mg/ml]													
	0.003	0.007	0.01	0.015	0.03	0.1	0.3	0.45	0.6	1.0	1.25	1.5	1.75	2
	area covered by cells in percent compared to controls													
ASMP-MG7274	100	100	100	100	100	75	50	25	25	25	0	0	0	0
ASMP-TM7279	100	100	100	100	100	100	75	50	25	0	0	0	0	0
ASMP-CA9656	100	100	100	100	100	100	100	100	100	75	50	25	25	0
ANMP-MG7274	100	100	100	100	100	100	100	100	100	75	75	50	25	0
ANMP-TM7279	100	100	100	100	100	100	100	100	100	75	50	25	25	0
ANMP-CA9656	100	100	100	100	100	100	100	100	100	75	75	50	25	0
AEMP-MG7274	100	100	100	100	100	100	75	75	50	25	0	0	0	0
AEMP-TM7279	100	100	100	100	100	100	100	100	100	75	75	50	25	0
AEMP-CA9656	100	100	100	100	100	100	100	100	100	75	50	25	0	0

Table 3: Concentration of different antigenic fractions resulting in 50% growth inhibition of keratinocyte cells (HaCaT cells)

strain	concentration of antigenic fraction [mg/ml]		
	ASMP	ANMP	AEMP
MG7274	0.3	1.5	0.6
TM7279	0.45	1.25	1.5
CA9656	1.25	1.5	1.25

Table 4: Influence of different antigenic fractions on cell proliferation of horse lymphocytes

concentration of antigenic fractions [µg/ml]	horse lymphocyte proliferation in % (controls without exposure to antigenic fractions = 100%)					
	ASMP-MG7274	ASMP-TM7279	ASMP-CA9656	AEMP-MG7274	AEMP-TM7279	AEMP-CA9656
500	81.5	36.2	37.8	19.4	7.0	1.08
50	117	106.9	109.8	76.1	10.2	46.9
5	181	172.8	119.8	129.7	47.3	138.3
0.5	98.6	93.8	134.1	147.8	133.3	138.3
0.05	271.3	94.3	181.5	143.5	94.8	149.8
0.005	146.7	207.6	144.7	104.7	109.9	146.3

Table 5A: Reaction of test animals (white mice, body weight 12-14g) after "first injection" of individual antigenic fractions

antigenic fraction	concentration [mg/ml] or [%(v/v)]	injection volume [ml]	number of animals vaccinated	number of animals showing					
				local pain	local reaction	increase of local temperature	loss of appetite	Impair-ment of loco-motion	lethal reactions
ASMP-MG7274	10.5mg/ml	0.5	10	0	0	0	0	0	0
ASMP-TM7279	12.5mg/ml	0.5	10	0	0	0	0	0	0
ASMP-CA9656	15.5mg/ml	0.5	9	0	0	0	0	0	0
ANMP-MG7274	3.3%	0.5	8	0	1	0	0	0	0
ANMP-TM7279	3.3%	0.5	10	0	2	0	0	0	0
ANMP-CA9656	3.3%	0.5	10	0	1	0	0	0	0
AEMP-MG7274	15.5mg/ml	0.5	10	0	1	0	0	0	0
AEMP-TM7279	11.3mg/ml	0.5	10	0	1	0	0	0	0
AEMP-CA9656	13.5mg/ml	0.5	10	0	1	0	0	0	0

Table 5B: Reaction of test animals after "first injection" of complex preparations

complex	No	concentration [mg/ml] or [%(v/v)]	injection volume [ml]	number of animals vaccinated	number of animals showing					
					local pain	local reaction	increase of local temperature	loss of appetite	Impair-ment of loco-motion	lethal reactions
white mice (body weight 12-14g)										
ANMP-MG7274 ANMP-TM7279 AEMP-TM7279	4.2	2.5% 2.5% 10.5mg/ml	0.5	10	0	0	0	0	0	0
ANMP-CA9656 AEMP-TM7279	4.1	2.5% 7.1mg/ml	0.5	10	0	0	0	0	0	0
ANMP-MG7274 ANMP-TM7279 ASMP-CA9656	5	1.75% 1.75% 15.6mg/ml	0.5	10	0	0	0	0	0	0
Guinea pigs (body weight 150-200g)										
ANMP-MG7274 ANMP-TM7279 AEMP-TM7279	4.2	2.5% 2.5% 10.5mg/ml	0.5	5	0	0	0	0	0	0
ANMP-CA9656 AEMP-TM7279	4.1	2.5% 7.1mg/ml	0.5	5	0	0	0	0	0	0
ANMP-MG7274 ANMP-TM7279 ASMP-CA9656	5	1.75% 1.75% 15.6mg/ml	0.5	5	0	0	0	0	0	0

Table.6A: Reaction of test animals (white mice, body weight 12-14g) after "second injection" of individual antigenic fractions

antigenic fraction	concentration [mg/ml] or [%(v/v)]	injection volume [ml]	number of animals vaccinated	number of animals showing					
				local pain	local reaction	increase of local temperature	loss of appetite	Impair-ment of loco-motion	lethal reactions
ASMP-MG7274	10.5mg/ml	1.0	10	0	0	0	0	0	0
ASMP-TM7279	12.5mg/ml	1.0	10	0	0	0	0	0	0
ASMP-CA9656	15.5mg/ml	1.0	9	0	0	0	0	0	0
ANMP-MG7274	3.3%	0.5	8	0	1	0	0	0	0
ANMP-TM7279	3.3%	0.5	10	0	2	0	0	0	0
ANMP-CA9656	3.3%	1.0	10	0	1	0	0	0	0
AEMP-MG7274	15.5mg/ml	0.5	10	0	1	0	0	0	0
AEMP-TM7279	11.3mg/ml	1.0	10	0	1	0	0	0	0
AEMP-CA9656	13.5mg/ml	0.5	10	0	1	0	0	0	0

Table 6B: Reaction of test animals after "second injection" of complex preparations

complex	No	concentration [mg/ml] or [%(v/v)]	injection volume [ml]	number of animals vaccinated	number of animals showing					
					local pain	local reaction	increase of local temperature	loss of appetite	Impair-ment of loco-motion	lethal reactions
white mice (body weight 12-14g)										
ANMP-MG7274 ANMP-TM7279 AEMP-TM7279	4.2	2.5% 2.5% 10.5mg/ml	0.5	10	0	0	0	0	0	0
ANMP-CA9656 AEMP-TM7279	4.1	2.5% 7.1mg/ml	0.5	10	0	0	0	0	0	0
ANMP-MG7274 ANMP-TM7279 ASMP-CA9656	5	1.75% 1.75% 15.6mg/ml	0.5	10	0	0	0	0	0	0
Guinea pigs (body weight 150-200g)										
ANMP-MG7274 ANMP-TM7279 AEMP-TM7279	4.2	2.5% 2.5% 10.5mg/ml	1.0	5	0	0	0	0	0	0
ANMP-CA9656 AEMP-TM7279	4.1	2.5% 7.1mg/ml	1.0	5	0	0	0	0	0	0
ANMP-MG7274 ANMP-TM7279 ASMP-CA9656	5	1.75% 1.75% 15.6mg/ml	1.0	5	0	0	0	0	0	0

Table 7: Reaction of horses after injection (single injection) of complex preparations (each horse received complexes No. 4.1, 4.2 and 5 at the same time in separate injections at separate locations)

complex	No	concentration [mg/ml] or [%(v/v)]	injection volume [ml]	number of horses vaccinated	number of horses with local reactions		number of horses with general reactions:			
					pain	oedema/ inflammation	increase of temperature	loss of appetite	Impair-ment of loco-motion	loss of animals
ANMP-MG7274	4.2	3.0%	0.5	3	0	0	0	0	0	0
ANMP-TM7279		3.0%								
AEMP-TM7279		18.5mg/ml								
ANMP-CA9656	4.1	3.0%	0.5		0	0				
AEMP-TM7279		15.6								
ANMP-MG7274	5	3.0%	0.5		0	0				
ANMP-TM7279		3.0%								
ASMP-CA9656		15.6								

Table 8: Condition of skin and hairy coat after injection of complex antigenic preparations in white mice (body weight 12-14g)

complex	No	concentration [mg/ml] or [%(v/v)]	injection [ml]		number of animals	number of animals showing the following condition of the skin after vaccination:		number of animals showing the following condition of the hairy coat after vaccination:	
			first	second		scaling	smooth	smooth and shining	touseling and dim
ANMP-MG7274 ANMP-TM7279 AEMP-TM7279	4.2	2.5% 2.5% 10.5mg	0.5	1.0	2	0	2	2	0
ANMP-CA9656 AEMP-TM7279	4.1	2.5% 7.1mg	0.5	1.0	3	0	3	3	0
ANMP-MG7274 ANMP-TM7279 ASMP-CA9656	5	1.75% 1.75% 15.6mg	0.5	1.0	1	0	1	1	0
not vaccinated	-	-	-	-	3	3	0	2	1

Table 9: Efficacy of vaccination with different complex preparations determined in Iceland horses afflicted with Summer Eczema

complex	No	concentration [mg/ml] or [%(v/v)]	injection volume [ml]	number of injections	number of horses vaccinated	four weeks after third vaccination: number of horses	
						cured	not cured
						from Summer Eczema	
ASMP-MG7274 ASMP-TM7279 ASMP-CA9656	1	10 mg/ml 10 mg/ml 10 mg/ml	1	3	3	3	0
ANMP-MG7274 ANMP-TM7279 ANMP-CA9656	2	1% 1% 1%	1	3	3	0	3
AEMP-MG7274 AEMP-TM7279 AEMP-CA9656	3	10 mg/ml 10 mg/ml 10 mg/ml	1	3	3	1	2

Table 10: Efficacy of vaccination with different complex preparations, determined in Iceland horses afflicted with Summer Eczema

complex	No	concentration of individual fractions [mg/ml] or [%(v/v)]	injection volume [ml]	number of injections	number of horses vaccinated	four weeks after third injection: number of horses cured from	
						itching	eczema
ASMP-MG7274 ASMP-TM7279 ASMP-CA9656	1	10mg/ml 10mg/ml 10mg/ml	1	3	3	3	3
ANMP-MG7274 ANMP-TM7279 ANMP-CA9656	2	1 % 1 % 1 %	1	3	3	1	0
AEMP-MG7274 AEMP-TM7279 AEMP-CA9656	3	10mg/ml 10mg/ml 10mg/ml	1	3	3	2	1
no antigen (Carrier A only)	-	- (control)	1	3	3	0	0

Table 11: Safety of vaccination with different antigenic preparations determined in Iceland horses afflicted with Summer Eczema

complex	No	concentration [mg/ml] or [%(v/v)]	injection volume [ml]	number of injections	number of horses vaccinated	number of horses with local side effects observed 1 to 3 days after first to third injection		number of horses with general side effects observed 1 to 3 days after first to third injection	
						swelling	pain	fever	loss of appetite
ASMP-MG7274 ASMP-TM7279 ASMP-CA9656	1	10mg/ml 10mg/ml 10mg/ml	1	3	3	0	0	0	0
ANMP-MG7274 ANMP-TM7279 ANMP-CA9656	2	1 % 1 % 1 %	1	3	3	0	0	0	0
AEMP-MG7274 AEMP-TM7279 AEMP-CA9656	3	10mg/ml 10mg/ml 10mg/ml	1	3	3	1	0	0	0
no antigen (Carrier A only)	-	- (control)	1	3	3	0	0	0	0

Table 12: NMR-Spectra

antigenic preparations	acetate (s)	douplet amino acids	triplet amino acids	multiplet carbohadrates	isolated CH2 amino acids	endstanding alcyl-CH amino acids	aryl-H amino acids
ASMP							
MG 7274/ 9-18-1 (Figure 4)	1.92ppm	CH3 (d,6.8 Hz) 1.33ppm	CH3 (t,7.1 Hz) 1,18ppm	3.2 - 4.3 ppm	1.7 - 3.45ppm	0.95ppm	
		CH3 (d,7.5 Hz) 1.48ppm		4.9 - 5.4 ppm			
					CH2(AcB,16Hz) 2.7ppm/2.5ppm		
CA 9656/ b008 (Figure 2)	1.92ppm	CH3 (d,7.0Hz) 1,33ppm	CH3 (t,7.0Hz) 1,18ppm	3.4-4.6 ppm	3.28 ppm	0.95 ppm	
		CH3 (d,7.3HZ) 1,48ppm		4.9-5.24 ppm			
TM 7279/ 32-m-1-5 (Figure 3)	1.92 ppm	CH3 (d, 7.1 Hz) 1,33ppm	CH3 (t, 7.1 Hz) 1,18ppm	3.5 - 4.35ppm	2.1 - 3.3 ppm	0.85 - 0.95 ppm	
		CH3 (d, 7.1 Hz) 1,48ppm		5.0 - 5.25 ppm			
AEMP							
TM 7279/ p32-5-1 (Figure 1, A-C)		CH3 (d, 6.5 Hz) 1,33 ppm		3.2 - 4.07 ppm	1.6 - 3.12 ppm		
		CH3 (d, 7.5 Hz) 1,48 ppm				0,84 - 1.08 ppm	7.15 - 7.9 ppm
				CH (d, 8.2Hz) 4.65 ppm			
				CH (d, 4.0Hz) 5.23 ppm			

ppm = part per million

s = singulet

Figures 1 to 4:

NMR experiments of ASMP and AEMP fractions as shown in Figures 1 to 4 were performed according to the following:

Spectra have been obtained in D_2O on a 250MHZ BRUKER digital NMR-spectrometer (model DRX 400) with a ^1H-frequency of 400.13 Mc. Sweep width is 14.5 ppm, ambient temperature is 300K. Chemical shifts are referenced by means of the solvent distance.

The standard ^1H-one dimensional spectra have been obtained using the appropriate BRUKER pulse program.

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BUDAPESTER VERTRAG ÜBER DIE INTERNATIONALE ANERKENNUNG DER HINTERLEGUNG VON MIKROORGANISMEN FÜR DIE ZWECKE VON PATENTVERFAHREN

ERKLÄRUNG BEI ERSTHINTERLEGUNG
(REGEL 6.1)

An
DSM-DEUTSCHE SAMMLUNG VON
MIKROORGANISMEN UND ZELLKULTUREN GmbH
Mascheroder Weg 1b
D-3300 Braunschweig

Von der Hinterlegungsstelle auszufüllen:
DSM-Aufnahmenummer: 7274
Eingangsdatum der Kultur: Eingegangen/Received 1992-10-01 Deutsche Sammlung von Mikroorganismen

BAKTERIEN/PILZE[1]

DER UNTERZEICHNETE HINTERLEGT DEN NACHSTEHEND BEZEICHNETEN MIKROORGANISMUS AUFGRUND DES BUDAPESTER VERTRAGES UND VERPFLICHTET SICH, DIE HINTERLEGUNG WÄHREND DER IN REGEL 9.1 GENANNTEN DAUER NICHT ZURÜCKZUNEHMEN[2]

I. KENNZEICHNUNG DES MIKROORGANISMUS

Bezugszeichen[3]: No. 59
Microsporum gypseum (Bodin) Guiart et Grigorakis, 1928
Taxonomische Bezeichnung[4]:
classis Deuteromycetes
ordo Hyphomycetales
familia Mucedinaceae

Die zu hinterlegende Kultur ist:
(+) eine Reinkultur
( ) eine Mischkultur
(Zutreffendes bitte ankreuzen)

II. ZÜCHTUNGSBEDINGUNGEN ( )[5]

Medium:
beer-wort agar 7°B

pH vor der Sterilisation 7,5-7,8
Sterilisation 15 min bei 121 °c
pH nach Sterilisation: 6,3-6,9
Verhalten gegenüber Sauerstoff:
(+) aerob
( ) mikroaerophil
( ) obligat anaerob
Besondere Ansprüche an die Gasatmosphäre:

Bebrütungstemperatur: 26-28 °c
Bebrütungsdauer: 10-15 days
Aufbewahrung bei: +2-8 °c
Überimpfungsintervall: 3-4 month

[1] Die DSM nimmt zur Hinterlegung ausschließlich Mikroorganismen an, die gemäß Merkblatt 'Sichere Biotechnologie: Bakterien' (B 006 1/92 ZH 1/346) der Berufsgenossenschaft der chemischen Industrie zu den Risikogruppen 1 oder 2 gehören, bzw. unter Anwendung der Laborsicherheitsmaßnahmen L1 oder L2 entsprechend dem "Gesetz zur Regelung von Fragen der Gentechnik" (BGBL I, pp. 1080; 23/06/90) bearbeitet werden können.

[2] Dieses Formblatt kann auch verwendet werden, wenn der Unterzeichnete eine Hinterlegung eines Mikroorganismus, die er selbst oder sein Rechtsvorgänger außerhalb des Budapester Vertrags bei derselben Hinterlegungsstelle vorgenommen hat, in eine Hinterlegung nach dem Budapester Vertrag umwandeln. Dabei ist unerheblich, ob die Ersthinterlegung vor (Regel 6.4 Buchstabe d) oder nach dem Zeitpunkt stattgefunden hat, zu dem die Stelle den Status einer internationalen Hinterlegungsstelle erworben hat.

[3] Nummer, Symbole usw., die der Hinterleger dem Mikroorganismus zugeteilt hat.

[4] Es wird dringend empfohlen, die taxonomische Bezeichnung und/oder die wissenschaftliche Beschreibung des Mikroorganismus (siehe unter VII.) anzugeben.

[5] Ankreuzen, wenn auf einem besonderen Blatt weitere Angaben eingereicht werden.

III. AUFBEWAHRUNGSBEDINGUNGEN ( )[5]

The strain is stored in liophylized condition in ampulles under vacuum or on agar nutrient medium in tubes at +2-8°C.

IV. BEDINGUNGEN FÜR DIE LEBENSFÄHIGKEITSPRÜFUNG ( )[5]

With sterile pipette 3 cc of sterile phisiological solution are added to liophylised fungal material for full resuspension of culture. Fungal suspension is seeded on sloped agar and it is cultivated during 15-20 days at +26-28°C. The culture from a tube is seeded on new sloped agar by spore suspension and it is cultivated during 10-15 days

V. BESTANDTEILE DER MISCHKULTUR (wenn zutreffend) ( )[5]

Beschreibung der Bestandteile:

Verfahren, die die Prüfung auf Lebensfähigkeit der Bestandteile ermöglichen:

VI. EIGENSCHAFTEN, DIE FÜR DIE GESUNDHEIT ODER DIE UMWELT GEFÄHRLICH SIND ( )[5]

Risikogruppe des unter I. genannten Mikroorganismus gemäß Merkblatt "Sichere Biotechnologie: Bakterien" (B 006 1/92 ZH/346) der Berufsgenossenschaft der chemischen Industrie[1]:

.....

Der zu hinterlegende Mikroorganismus darf unter folgenden Laborsicherheitsmaßnahmen[1] gehandhabt werden:

( ) L1 ( ) L2

Der Mikroorganismus hat folgende Eigenschaften, die für die Gesundheit oder die Umwelt gefährlich sind oder sein können:

Bitte angeben: The strain is weak-virulent. The result 10-12 days after application of a dose of 500-600 thousand cells of fungal matter per cm^2 to the scarified skin of a rabbit necrotic scabs are formed. Spontaneous recovery following 22 days.

( ) **Dem Unterzeichneten sind derartige Eigenschaften nicht bekannt.**

[1] Die DSM nimmt zur Hinterlegung ausschließlich Mikroorganismen an, die gemäß Merkblatt "Sichere Biotechnologie: Bakterien" (B 006 1/92 ZH 1/346) der Berufsgenossenschaft der chemischen Industrie zu den Risikogruppen 1 oder 2 gehören, bzw. unter Anwendung der Laborsicherheitsmaßnahmen L1 oder L2 entsprechend dem "Gesetz zur Regelung von Fragen der Gentechnik" (BGBL I, pp. 1080; 22/06/90) bearbeitet werden können.

[5] Ankreuzen, wenn auf einem gesonderten Blatt weitere Angaben eingereicht werden.

VIII. WISSENSCHAFTLICHE BESCHREIBUNG[7] ( )[5]

Mature 10-15 day colony is white, velvety-fluffy, flat with slight elevation in centre of colony, growing margin fringed, undersurface brownish, diameter of colony 25-30 mm. Septate branching hyphae 2-3 um wide, numerous oval, pyriform, cylindrical microconidia measuring 2-4x3-6 um, non or few macroconidia, elliptical, stretched-oval shape with 2-5 septates, measuring 7-15x25-40 um.
The strain was obtained by means of directed selection based on spore production and attenuation of epizootic strain.

IX. WEITERE ANGABEN ( )[8]

The strain was isolated from a horse in 1985 (Russia).
The strain was deposited at the Collection of Pathogenic Fungi within the Russian Ministry of Health Centre for Deep Mycoses in Sankt-Peterburg, No.729/59.

X. HINTERLEGER[9]

Name: Dr. L.G.Ivanova
Dr. I.D.Polyakov
BOEHRINGER INGELHEIM VETMEDICA GMBH
pp a.
D.Janott E. Richter

Anschrift: Russia, Moscow 115682
Zadonsky proezd, 24-1-142

Unterschrift:

Datum: 10.09.1992.

5 Ankreuzen, wenn auf einem gesonderten Blatt weitere Angaben eingereicht werden.
7 Es wird dringend empfohlen, die wissenschaftliche Beschreibung und/oder die vorgeschlagene taxonomische Bezeichnung (unter I.) des Mikroorganismus anzugeben.
8 Ankreuzen, wenn auf einem gesonderten Blatt weitere Angaben (außer den unter Fußnote 5 genannten) eingereicht werden, z. B. Herkunft des Mikroorganismus, Name und Anschrift anderer Hinterlegungsstellen, bei denen der Mikroorganismus hinterlegt worden ist, oder das für die Festlegung der vorgeschlagenen taxonomischen Bezeichnung angewandte Kriterium. (Diese Angaben sind fakultativ).
9 Der Name des Hinterlegers muß mit der Unterschrift identisch sein. Bei Firmen müssen zwei offizielle Firmenvertreter unterschreiben. Wenn natürliche Personen für eine juristische Person unterzeichnen, so ist deren Unterschrift in Maschinenschrift zu wiederholen.

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BUDAPESTER VERTRAG ÜBER DIE INTERNATIONALE

ANERKENNUNG DER HINTERLEGUNG VON MIKROORGANISMEN

FÜR DIE ZWECKE VON PATENTVERFAHREN

ERKLÄRUNG BEI ERSTHINTERLEGUNG
(REGEL 6.1)

An
DSM-DEUTSCHE SAMMLUNG VON
MIKROORGANISMEN UND ZELLKULTUREN GmbH
Mascheroder Weg 1b
D-3300 Braunschweig

Von der Hinterlegungsstelle auszufüllen:
DSM-Aufnahmenummer: 7275
Eingangsdatum der Kultur: Eingegangen/Received 1992-10-01 Deutsche Sammlung von Mikroorganismen

BAKTERIEN/PILZE[1]

DER UNTERZEICHNETE HINTERLEGT DEN NACHSTEHEND BEZEICHNETEN MIKROORGANISMUS AUFGRUND DES BUDAPESTER VERTRAGES UND VERPFLICHTET SICH, DIE HINTERLEGUNG WÄHREND DER IN REGEL 9.1 GENANNTEN DAUER NICHT ZURÜCKZUNEHMEN[2]

I. KENNZEICHNUNG DES MIKROORGANISMUS

Bezugszeichen[3]: No. I20
Taxonomische Bezeichnung[4]: Microsporum canis var.distortum (Di Menna et Marples)Matsumoto, Padhye et Ajello,1983
classis Deuteromycetes
ordo Hyphomycetales
familia Mucedinaceae

Die zu hinterlegende Kultur ist:
(+) eine Reinkultur
( ) eine Mischkultur
(Zutreffendes bitte ankreuzen)

II. ZÜCHTUNGSBEDINGUNGEN ( )[5]

Medium:
beer-wort agar 7°B.

pH vor der Sterilisation 7,5-7,8
Sterilisation I5 min bei I2I °C
pH nach Sterilisation: 6,3-6,9
Verhalten gegenüber Sauerstoff:
(+) aerob
( ) mikroaerophil
( ) obligat anaerob
Besondere Ansprüche an die Gasatmosphäre:

Bebrütungstemperatur: 26-28 °C
Bebrütungsdauer: I0-I5 days
Aufbewahrung bei: +2-8 °C
Überimpfungsintervall: 3-4 month.

1 Die DSM nimmt zur Hinterlegung ausschließlich Mikroorganismen an, die gemäß Merkblatt 'Sichere Biotechnologie: Bakterien' (B 006 1/92 ZH 1/346) der Berufsgenossenschaft der chemischen Industrie zu den Risikogruppen 1 oder 2 gehören, bzw. unter Anwendung der Laborsicherheitsmaßnahmen L1 oder L2 entsprechend dem "Gesetz zur Regelung von Fragen der Gentechnik" (BGBL I, pp. 1080; 23/06/90) bearbeitet werden können.

2 Dieses Formblatt kann auch verwendet werden, wenn der Unterzeichnete eine Hinterlegung eines Mikroorganismus, die er selbst oder sein Rechtsvorgänger außerhalb des Budapester Vertrags bei derselben Hinterlegungsstelle vorgenommen hat, in eine Hinterlegung nach dem Budapester Vertrag umwandelt. Dabei ist unerheblich, ob die Ersthinterlegung vor (Regel 6.4 Buchstabe d) oder nach dem Zeitpunkt stattgefunden hat, zu dem die Stelle den Status einer internationalen Hinterlegungsstelle erworben hat.

3 Nummer, Symbole usw., die der Hinterleger dem Mikroorganismus zugeteilt hat.

4 Es wird dringend empfohlen, die taxonomische Bezeichnung und/oder die wissenschaftliche Beschreibung des Mikroorganismus (siehe unter VII.) anzugeben.

5 Ankreuzen, wenn auf einem besonderen Blatt weitere Angaben eingereicht werden.

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III. AUFBEWAHRUNGSBEDINGUNGEN ( )

The strain is stored in liophylized condition in ampulles under vacuum or on agar nutrient medium in tubes at +2-8°C.

IV. BEDINGUNGEN FÜR DIE LEBENSFÄHIGKEITSPRÜFUNG ( )[5]

With sterile pipette 3 cc of sterile phisiological solution are added to liophilised fungal material for full resuspension of culture. Fungal suspension is seeded on sloped agar and it is cultivated during 15-20 days at +26-28°C.
The cultire from a tube is seeded on new sloped agar by spore suspension and it is cultivated during 10-15 days at 26-28°C.

V. BESTANDTEILE DER MISCHKULTUR (wenn zutreffend) ( )[5]

Beschreibung der Bestandteile:

Verfahren, die die Prüfung auf Lebensfähigkeit der Bestandteile ermöglichen:

VI. EIGENSCHAFTEN, DIE FÜR DIE GESUNDHEIT ODER DIE UMWELT GEFÄHRLICH SIND ( )[5]

Risikogruppe des unter I. genannten Mikroorganismus gemäß Merkblatt 'Sichere Biotechnologie: Bakterien' (B 006 1/92 ZH/346) der Berufsgenossenschaft der chemischen Industrie[1]:

.....

Der zu hinterlegende Mikroorganismus darf unter folgenden Laborsicherheitsmaßnahmen[1] gehandhabt werden:

( ) L1 ( ) L2

Der Mikroorganismus hat folgende Eigenschaften, die für die Gesundheit oder die Umwelt gefährlich sind oder sein können:

Bitte angeben: The strain is weak-virulent. The result 10-12 days after application of a dose of 500-600 thousand cells of fungal matter per cm^2 to the scarified skin of a rabbit necrotic scabs are formed. Spontaneous recovery following 22-25 days.

( ) Dem Unterzeichneten sind derartige Eigenschaften nicht bekannt.

[1] Die DSM nimmt zur Hinterlegung ausschließlich Mikroorganismen an, die gemäß Merkblatt 'Sichere Biotechnologie: Bakterien' (B 006 1/92 ZH 1/346) der Berufsgenossenschaft der chemischen Industrie zu den Risikogruppen 1 oder 2 gehören, bzw. unter Anwendung der Laborsicherheitsmaßnahmen L1 oder L2 entsprechend dem "Gesetz zur Regelung von Fragen der Gentechnik" (BGBL I, pp. 1080; 23/06/90) bearbeitet werden konnen.

[5] Ankreuzen, wenn auf einem gesondertes Blatt weitere Angaben eingereicht werden.

VIII. WISSENSCHAFTLICHE BESCHREIBUNG[7] ( )[5]

Mature 10-15 day colony is cream velvety-powdered, bitte-like elevation in centre, growing margin strait, finely-fringed, undersurface light-brown with dark-brown centre, diameter of colony 25-30 mm. Septate branching hyphae 1-3 um wide, numerous pyriform, oval, cylindrical microconidia measuring 1-3x3-8 um, few irregular deformed macroconidia distorted or fusiform with 2-9 septates, measuring 8-20x 25-70 um. The strain was obtained means of directed selection based on spore production and attenuation of epizootic str

IX. WEITERE ANGABEN ( )[8]

The strain was isolated from a black panther in 1987(Russia). The strain was deposited at the Collection of Pathogenic Fungi within the Russian Ministry of Health Centre for Deep Mycoses in Sankt-Peterburg, No. VKPG F-728/120.

X. HINTERLEGER[9]

Name: Dr. L.G.Ivanova
Dr. I.D.Polyakov
BOEHRINGER INGELHEIM VETMEDICA GMBH
ppa.
D. Janott Dr.E Richter

Unterschrift:

Anschrift: Russia, Moscow 115682
Zadonsky proezd, 24-1-142

Datum: 10.09.1992

5 Ankreuzen, wenn auf einem gesonderten Blatt weitere Angaben eingereicht werden.
7 Es wird dringend empfohlen, die wissenschaftliche Beschreibung und/oder die vorgeschlagene taxonomische Bezeichnung (unter I.) des Mikroorganismus anzugeben.
8 Ankreuzen, wenn auf einem gesonderten Blatt weitere Angaben (außer den unter Fußnote 5 genannten) eingereicht werden, z. B. Herkunft des Mikroorganismus, Name und Anschrift anderer Hinterlegungsstellen, bei denen der Mikroorganismus hinterlegt worden ist, oder das für die Festlegung der vorgeschlagenen taxonomischen Bezeichnung angewandte Kriterium. (Diese Angaben sind fakultativ).
9 Der Name des Hinterlegers muß mit der Unterschrift identisch sein. Bei Firmen müssen zwei offizielle Firmenvertreter unterschreiben. Wenn natürliche Personen für eine juristische Person unterzeichnen, so ist deren Unterschrift in Maschinenschrift zu wiederholen.

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BUDAPESTER VERTRAG ÜBER DIE INTERNATIONALE ANERKENNUNG DER HINTERLEGUNG VON MIKROORGANISMEN FÜR DIE ZWECKE VON PATENTVERFAHREN

ERKLÄRUNG BEI ERSTHINTERLEGUNG
(REGEL 6.1)

An
DSM-DEUTSCHE SAMMLUNG VON
MIKROORGANISMEN UND ZELLKULTUREN GmbH
Mascheroder Weg 1b
D-3300 Braunschweig

Von der Hinterlegungsstelle auszufüllen:
DSM-Aufnahmenummer: 7276
Eingangsdatum der Kultur: Eingegangen/Received 1992-10-01 Deutsche Sammlung von Mikroorganismen

BAKTERIEN/PILZE[1]

DER UNTERZEICHNETE HINTERLEGT DEN NACHSTEHEND BEZEICHNETEN MIKROORGANISMUS AUFGRUND DES BUDAPESTER VERTRAGES UND VERPFLICHTET SICH, DIE HINTERLEGUNG WÄHREND DER IN REGEL 9.1 GENANNTEN DAUER NICHT ZURÜCKZUNEHMEN[2]

I. KENNZEICHNUNG DES MIKROORGANISMUS

Bezugszeichen[3]: No. 38I

Trichophyton equinum (Matruchot et Dassonville)Gedoelst, I902

Taxonomische Bezeichnung[4]:
classis Deuteromycetes
ordo Hyphomycetales
familia Mucedinaceae

Die zu hinterlegende Kultur ist:
(+) eine Reinkultur
( ) eine Mischkultur
(Zutreffendes bitte ankreuzen)

II. ZÜCHTUNGSBEDINGUNGEN ( )[5]

Medium:
beer-wort. agar 7°B

pH vor der Sterilisation 7,5-7,8
Sterilisation I5 min bei I2I °C
pH nach Sterilisation: 6,3-6,9
Verhalten gegenüber Sauerstoff:
(+) aerob
( ) mikroaerophil
( ) obligat anaerob
Besondere Ansprüche an die Gasatmosphäre:

Bebrütungstemperatur: 26-28 °C
Bebrütungsdauer: I0-I5 days
Aufbewahrung bei: +2-8 °C
Überimpfungsintervall: 3-4 month

[1] Die DSM nimmt zur Hinterlegung ausschließlich Mikroorganismen an, die gemäß Merkblatt 'Sichere Biotechnologie: Bakterien' (B 006 1/92 ZH 1/346) der Berufsgenossenschaft der chemischen Industrie zu den Risikogruppen 1 oder 2 gehören, bzw. unter Anwendung der Laborsicherheitsmaßnahmen L1 oder L2 entsprechend dem "Gesetz zur Regelung von Fragen der Gentechnik" (BGBL I, pp. 1080; 23/06/90) bearbeitet werden können.

[2] Dieses Formblatt kann auch verwendet werden, wenn der Unterzeichnete eine Hinterlegung eines Mikroorganismus, die er selbst oder sein Rechtsvorgänger außerhalb des Budapester Vertrags bei derselben Hinterlegungsstelle vorgenommen hat, in eine Hinterlegung nach dem Budapester Vertrag umwandelt. Dabei ist unerheblich, ob die Ersthinterlegung vor (Regel 6.4 Buchstabe d) oder nach dem Zeitpunkt stattgefunden hat, zu dem die Stelle den Status einer internationalen Hinterlegungsstelle erworben hat.

[3] Nummer, Symbole usw., die der Hinterleger dem Mikroorganismus zugeteilt hat.

[4] Es wird dringend empfohlen, die taxonomische Bezeichnung und/oder die wissenschaftliche Beschreibung des Mikroorganismus (siehe unter VII.) anzugeben.

[5] Ankreuzen, wenn auf einem besonderen Blatt weitere Angaben eingereicht werden.

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III. AUFBEWAHRUNGSBEDINGUNGEN ( )[5]

The strain is stored in liophylized condition in ampuiles under vacuum or on agar nutrient medium in tubes at +2-8°C.

IV. BEDINGUNGEN FÜR DIE LEBENSFÄHIGKEITSPRÜFUNG ( )[5]

With sterile pipette 3 cc of sterile phisiological solution are added to liophylized fungal matter for full resuspension of culture. Fungal suspension is seeded on sloped agar and it is cultivated during I0-I5 days at +26-28°C.
The culture from a tube is seeded on new sloped agar by spore suspension and it is cultivated during I0-I5 days at +26-28°C.

V. BESTANDTEILE DER MISCHKULTUR (wenn zutreffend) ( )[5]

Beschreibung der Bestandteile:

Verfahren, die die Prüfung auf Lebensfähigkeit der Bestandteile ermöglichen:

VI. EIGENSCHAFTEN, DIE FÜR DIE GESUNDHEIT ODER DIE UMWELT GEFÄHRLICH SIND ( )[5]

Risikogruppe des unter I. genannten Mikroorganismus gemäß Merkblatt 'Sichere Biotechnologie: Bakterien' (B 006 1/92 ZH/346) der Berufsgenossenschaft der chemischen Industrie[1]:

Der zu hinterlegende Mikroorganismus darf unter folgenden Laborsicherheitsmaßnahmen[1] gehandhabt werden:

( ) L1 ( ) L2

Der Mikroorganismus hat folgende Eigenschaften, die für die Gesundheit oder die Umwelt gefährlich sind oder sein können:

Bitte angeben: The strain is weak-virulent. The result I0-I2 days after application of a dose of 500-600 thousand cells of fungal matter per cm^2 to the scarified skin of a rabbit necrotic scsbs are formed. Spontaneous recovery following 20-22 days.

( ) Dem Unterzeichneten sind derartige Eigenschaften nicht bekannt.

[1] Die DSM nimmt zur Hinterlegung ausschließlich Mikroorganismen an, die gemäß Merkblatt 'Sichere Biotechnologie: Bakterien' (B 006 1/92 ZH 1/346) der Berufsgenossenschaft der chemischen Industrie zu den Risikogruppen 1 oder 2 gehören, bzw. unter Anwendung der Laborsicherheitsmaßnahmen L1 oder L2 entsprechend dem "Gesetz zur Regelung von Fragen der Gentechnik" (BGBL I, pp. 1080; 23/06/90) bearbeitet werden können.

[5] Ankreuzen, wenn auf einem gesonderten Blatt weitere Angaben eingereicht werden.

VIII. WISSENSCHAFTLICHE BESCHREIBUNG[7] 66 ( )[5]

Mature 10-15 day colony is white velvety-powdered, flat with slight elevation in centre, growing margin strait, fringed, undersurface light-brown, diameter of colony 15-20 mm. Septate branching hyphae 1-3 um wide, numerous oval pyriform microconidia measuring 2-3x3-6 um, no macroconidia.
The strain was obtained by means of directed selection based on spore production and attenuation of epizootic strain.

IX. WEITERE ANGABEN ( )[8]

The strain was isolated from a horse in 1986 (Russia).
The strain was deposited at the Collection of Pathogenic Fungi within the Russian Ministry of Health Centre for Deep Mycoses in Sankt-Peterburg, No.VKPG F-929/381.

X. HINTERLEGER[9]

Name: Dr. L.G.Ivanova
Dr. I.D.Polyakov
BOEHRINGER INGELHEIM VETMEDICA GMBH
ODA.

Unterschrift: L. Ivanova

Anschrift: D. Janott Dr. E. Richter
Russia, Moscow 115682
Zadonsky proezd, 24-1-142

Datum: 10.09.1992.

[5] Ankreuzen, wenn auf einem gesonderten Blatt weitere Angaben eingereicht werden.
[7] Es wird dringend empfohlen, die wissenschaftliche Beschreibung und/oder die vorgeschlagene taxonomische Bezeichnung (unter I.) des Mikroorganismus anzugeben.
[8] Ankreuzen, wenn auf einem gesonderten Blatt weitere Angaben (außer den unter Fußnote 5 genannten) eingereicht werden, z. B. Herkunft des Mikroorganismus, Name und Anschrift anderer Hinterlegungsstellen, bei denen der Mikroorganismus hinterlegt worden ist, oder das für die Festlegung der vorgeschlagenen taxonomischen Bezeichnung angewandte Kriterium. (Diese Angaben sind fakultativ).
[9] Der Name des Hinterlegers muß mit der Unterschrift identisch sein. Bei Firmen müssen zwei offizielle Firmenvertreter unterschreiben. Wenn natürliche Personen für eine juristische Person unterzeichnen, so ist deren Unterschrift in Maschinenschrift zu wiederholen.

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BUDAPESTER VERTRAG ÜBER DIE INTERNATIONALE

ANERKENNUNG DER HINTERLEGUNG VON MIKROORGANISMEN

FÜR DIE ZWECKE VON PATENTVERFAHREN

ERKLÄRUNG BEI ERSTHINTERLEGUNG
(REGEL 6.1)

An
DSM-DEUTSCHE SAMMLUNG VON
MIKROORGANISMEN UND ZELLKULTUREN GmbH
Mascheroder Weg 1b
D-3300 Braunschweig

Von der Hinterlegungsstelle auszufüllen:
DSM-Aufnahmenummer: 7277
Eingangsdatum der Kultur: Eingegangen/Received 1992-10-01 Deutsche Sammlung von Mikroorganismen

BAKTERIEN/PILZE[1]

DER UNTERZEICHNETE HINTERLEGT DEN NACHSTEHEND BEZEICHNETEN MIKROORGANISMUS AUFGRUND DES BUDAPESTER VERTRAGES UND VERPFLICHTET SICH, DIE HINTERLEGUNG WÄHREND DER IN REGEL 9.1 GENANNTEN DAUER NICHT ZURÜCKZUNEHMEN[2]

I. KENNZEICHNUNG DES MIKROORGANISMUS

Bezugszeichen[3]: No. 410

Trichophyton verrucosum Bodin,1902

Taxonomische Bezeichnung[4]:
classis Deuteromycetes
ordo Hyphomycetales
familia Mucedinaceae

Die zu hinterlegende Kultur ist:
(+) eine Reinkultur
( ) eine Mischkultur
(Zutreffendes bitte ankreuzen)

II. ZÜCHTUNGSBEDINGUNGEN ( )[5]

Medium:
beer-wort agar 7°B

pH vor der Sterilisation 7,5-7,8
Sterilisation 15 min bei 121 °C
pH nach Sterilisation: 6,3-6,9
Verhalten gegenüber Sauerstoff:
(+) aerob
( ) mikroaerophil
( ) obligat anaerob
Besondere Ansprüche an die Gasatmosphäre:

Bebrütungstemperatur: 26-28 °C
Bebrütungsdauer: 15-20 days
Aufbewahrung bei: +2-8 °C
Überimpfungsintervall: 3-4 month

1 Die DSM nimmt zur Hinterlegung ausschließlich Mikroorganismen an, die gemäß Merkblatt 'Sichere Biotechnologie: Bakterien' (B 006 1/92 ZH 1/346) der Berufsgenossenschaft der chemischen Industrie zu den Risikogruppen 1 oder 2 gehören, bzw. unter Anwendung der Laborsicherheitsmaßnahmen L1 oder L2 entsprechend dem "Gesetz zur Regelung von Fragen der Gentechnik" (BGBL I, pp. 1080; 23/06/90) bearbeitet werden können.

2 Dieses Formblatt kann auch verwendet werden, wenn der Unterzeichnete eine Hinterlegung eines Mikroorganismus, die er selbst oder sein Rechtsvorgänger außerhalb des Budapester Vertrags bei derselben Hinterlegungsstelle vorgenommen hat, in eine Hinterlegung nach dem Budapester Vertrag umwandelt. Dabei ist unerheblich, ob die Ersthinterlegung vor (Regel 6.4 Buchstabe d) oder nach dem Zeitpunkt stattgefunden hat, zu dem die Stelle den Status einer internationalen Hinterlegungsstelle erworben hat.

3 Nummer, Symbole usw., die der Hinterleger dem Mikroorganismus zugeteilt hat.

4 Es wird dringend empfohlen, die taxonomische Bezeichnung und/oder die wissenschaftliche Beschreibung des Mikroorganismus (siehe unter VII.) anzugeben.

5 Ankreuzen, wenn auf einem besonderen Blatt weitere Angaben eingereicht werden.

( )[5]

The strain is stored in liophylized condition in ampulles under vacuum or on agar nutrient medium in tube at +2-8°C.

IV. BEDINGUNGEN FÜR DIE LEBENSFÄHIGKEITSPRÜFUNG ( )[5]

With sterile pipette 3 cc of sterile phisiological solution are added to liophylised fungal material for full resuspension of culture. Fungal suspension is seeded on sloped agar and it is cultivated during I5-20 days at +26-28°C.
The culture from a tube is seeded on new sloped agar by spore suspension, and it is cultivated during I5-20 days at +26-28°C.

V. BESTANDTEILE DER MISCHKULTUR (wenn zutreffend) ( )[5]

Beschreibung der Bestandteile:

Verfahren, die die Prüfung auf Lebensfähigkeit der Bestandteile ermöglichen:

VI. EIGENSCHAFTEN, DIE FÜR DIE GESUNDHEIT ODER DIE UMWELT GEFÄHRLICH SIND ( )[5]

Risikogruppe des unter I. genannten Mikroorganismus gemäß Merkblatt 'Sichere Biotechnologie: Bakterien' (B 006 1/92 ZH/346) der Berufsgenossenschaft der chemischen Industrie[1]:

.....

Der zu hinterlegende Mikroorganismus darf unter folgenden Laborsicherheitsmaßnahmen[1] gehandhabt werden:

( ) L1 ( ) L2

Der Mikroorganismus hat folgende Eigenschaften, die für die Gesundheit oder die Umwelt gefährlich sind oder sein können:

Bitte angeben: The strain is weak-virulent. The result I0-I2 days after application of a dose 500-600 thousand cells of fungal matter per cm^2 to the scarified skin of a rabbit necrotic scabs are formed. Spontaneous recovery following I9-20 days.

( ) **Dem Unterzeichneten sind derartige Eigenschaften nicht bekannt.**

[1] Die DSM nimmt zur Hinterlegung ausschließlich Mikroorganismen an, die gemäß Merkblatt 'Sichere Biotechnologie: Bakterien' (B 006 1/92 ZH 1/346) der Berufsgenossenschaft der chemischen Industrie zu den Risikogruppen 1 oder 2 gehören, bzw. unter Anwendung der Laborsicherheitsmaßnahmen L1 oder L2 entsprechend dem "Gesetz zur Regelung von Fragen der Gentechnik" (BGBL I, pp. 1080; 22/06/90) bearbeitet werden können.

[5] Ankreuzen, wenn auf einem gesonderten Blatt weitere Angaben eingereicht werden.

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VIII. WISSENSCHAFTLICHE BESCHREIBUNG[7] ( )[5]

Mature I5-20 days colony is white, velvety, convex, growing margin strait, undersurface colourless, diameter of colony 20-25 mm. Septate branching hyphae I-3 um wide, numerous oval, puriform microconidia measuring I,3-3,0x3,0-5,0 um, no macroconidia.
The strain was obtained by means of directed selection based on spore production and attenuation of epizootic strain.

IX. WEITERE ANGABEN ( )[8]

The strain was isolated from reindeer in I978 (Russia).
The strain was depcsited at the Collection of Pathogenic Fungi within the Russian Ministry of Health Centre for Deep Mycoses in Sankt-Peterburg, No.VKPG F-93I/4IO.

X. HINTERLEGER[9]

Name: Dr. L.G.Ivanova
Dr. I.D.Polyakov
BOEHRINGER INGELHEIM VETMEDICA GMBH
ppa.
D. Janott Dr. E. Richter

Unterschrift:

Anschrift: Russia, Moscow, II5682
Zadonsky proezd 24-I-I42

Datum: IO.09.I992.

5 Ankreuzen, wenn auf einem gesonderten Blatt weitere Angaben eingereicht werden.
7 Es wird dringend empfohlen, die wissenschaftliche Beschreibung und/oder die vorgeschlagene taxonomische Bezeichnung (unter I.) des Mikroorganismus anzugeben.
8 Ankreuzen, wenn auf einem gesonderten Blatt weitere Angaben (außer den unter Fußnote 5 genannten) eingereicht werden, z. B. Herkunft des Mikroorganismus, Name und Anschrift anderer Hinterlegungsstellen, bei denen der Mikroorganismus hinterlegt worden ist, oder das für die Festlegung der vorgeschlagenen taxonomischen Bezeichnung angewandte Kriterium. (Diese Angaben sind fakultativ).
9 Der Name des Hinterlegers muß mit der Unterschrift identisch sein. Bei Firmen müssen zwei offizielle Firmenvertreter unterschreiben. Wenn natürliche Personen für eine juristische Person unterzeichnen, so ist deren Unterschrift in Maschinenschrift zu wiederholen.

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BUDAPESTER VERTRAG ÜBER DIE INTERNATIONALE ANERKENNUNG DER HINTERLEGUNG VON MIKROORGANISMEN FÜR DIE ZWECKE VON PATENTVERFAHREN

ERKLÄRUNG BEI ERSTHINTERLEGUNG
(REGEL 6.1)

An
DSM-DEUTSCHE SAMMLUNG VON
MIKROORGANISMEN UND ZELLKULTUREN GmbH
Mascheroder Weg 1b
D-3300 Braunschweig

Von der Hinterlegungsstelle auszufüllen:
DSM-Aufnahmenummer: 7278
Eingangsdatum der Kultur: Eingegangen/Received 1992-10-01 Deutsche Sammlung von Mikroorganismen

BAKTERIEN/PILZE[1]

DER UNTERZEICHNETE HINTERLEGT DEN NACHSTEHEND BEZEICHNETEN MIKROORGANISMUS AUFGRUND DES BUDAPESTER VERTRAGES UND VERPFLICHTET SICH, DIE HINTERLEGUNG WÄHREND DER IN REGEL 9.1 GENANNTEN DAUER NICHT ZURÜCKZUNEHMEN[2]

I. KENNZEICHNUNG DES MIKROORGANISMUS

Bezugszeichen[3]: No.551
Trichophyton sarkisovii
Ivanova et Polyakov (1983)
Taxonomische Bezeichnung[4]:
classis Deuteromycetes
ordo Hyphomycetales
familia Mucedinaceae

Die zu hinterlegende Kultur ist:
(+) eine Reinkultur
( ) eine Mischkultur
(Zutreffendes bitte ankreuzen)

II. ZÜCHTUNGSBEDINGUNGEN ( )[5]

Medium:
beer-wort agar 7°B

pH vor der Sterilisation 7,5-7,8
Sterilisation 15 min bei 121 °c
pH nach Sterilisation: 6,3-6,9
Verhalten gegenüber Sauerstoff:
(+) aerob
( ) mikroaerophil
( ) obligat anaerob
Besondere Ansprüche an die Gasatmosphäre:

Bebrütungstemperatur: 26-28 °c
Bebrütungsdauer: 15-20 days
Aufbewahrung bei: +2-8 °c
Überimpfungsintervall: 3-4 month

1 Die DSM nimmt zur Hinterlegung ausschließlich Mikroorganismen an, die gemäß Merkblatt 'Sichere Biotechnologie: Bakterien' (B 006 1/92 ZH 1/346) der Berufsgenossenschaft der chemischen Industrie zu den Risikogruppen 1 oder 2 gehören, bzw. unter Anwendung der Laborsicherheitsmaßnahmen L1 oder L2 entsprechend dem "Gesetz zur Regelung von Fragen der Gentechnik" (BGBL I, pp. 1080; 23/06/90) bearbeitet werden können.

2 Dieses Formblatt kann auch verwendet werden, wenn der Unterzeichnete eine Hinterlegung eines Mikroorganismus, die er selbst oder sein Rechtsvorgänger außerhalb des Budapester Vertrags bei derselben Hinterlegungsstelle vorgenommen hat, in eine Hinterlegung nach dem Budapester Vertrag umwandelt. Dabei ist unerheblich, ob die Ersthinterlegung vor (Regel 6.4 Buchstabe d) oder nach dem Zeitpunkt stattgefunden hat, zu dem die Stelle den Status einer internationalen Hinterlegungsstelle erworben hat.

3 Nummer, Symbole usw., die der Hinterleger dem Mikroorganismus zugeteilt hat.

4 Es wird dringend empfohlen, die taxonomische Bezeichnung und/oder die wissenschaftliche Beschreibung des Mikroorganismus (siehe unter VII.) anzugeben.

5 Ankreuzen, wenn auf einem besonderen Blatt weitere Angaben eingereicht werden.

The strain is stored in liophylized condition in ampulles under vacuum or on agar nutrient medium in tubes at +2-8°C.

IV. BEDINGUNGEN FÜR DIE LEBENSFÄHIGKEITSPRÜFUNG ( )[5]

With sterile pipette 2 cc of sterile phisiological solution are added to liophylised fungal material for full resuspension of culture. Fungal suspension is seeded on sloped agar and it is cultivated during 15-20 days at +26-28°C. The culture from a tube is seeded on new sloped agar by spore suspension and it is cultivated during 15-20 days at +26-28°C.

V. BESTANDTEILE DER MISCHKULTUR (wenn zutreffend) ( )[5]

Beschreibung der Bestandteile:

Verfahren, die die Prüfung auf Lebensfähigkeit der Bestandteile ermöglichen:

VI. EIGENSCHAFTEN, DIE FÜR DIE GESUNDHEIT ODER DIE UMWELT GEFÄHRLICH SIND ( )[5]

Risikogruppe des unter I. genannten Mikroorganismus gemäß Merkblatt 'Sichere Biotechnologie: Bakterien' (B 006 1/92 ZH/346) der Berufsgenossenschaft der chemischen Industrie[1]:

.....

Der zu hinterlegende Mikroorganismus darf unter folgenden Laborsicherheitsmaßnahmen[1] gehandhabt werden:

( ) L1 ( ) L2

Der Mikroorganismus hat folgende Eigenschaften, die für die Gesundheit oder die Umwelt gefährlich sind oder sein können:

Bitte angeben: The strain is weak-virulent. The result 9-10 days after application of a dose 500-600 thousand cells of fungal matter per cm^2 to the scarified skin of a rabbit necrotic scabs are formed. Spontaneous recovery following 18-20 days.

( ) **Dem Unterzeichneten sind derartige Eigenschaften nicht bekannt.**

[1] Die DSM nimmt zur Hinterlegung ausschließlich Mikroorganismen an, die gemäß Merkblatt 'Sichere Biotechnologie: Bakterien' (B 006 1/92 ZH 1/346) der Berufsgenossenschaft der chemischen Industrie zu den Risikogruppen 1 oder 2 gehören, bzw. unter Anwendung der Laborsicherheitsmaßnahmen L1 oder L2 entsprechend dem "Gesetz zur Regelung von Fragen der Gentechnik" (BGBL I, pp. 1080; 23/06/90) bearbeitet werden können.

[5] Ankreuzen, wenn auf einem gesonderten Blatt weitere Angaben eingereicht werden.

VIII. WISSENSCHAFTLICHE BESCHREIBUNG[7] ( )[5]

Mature 15-20 day colony is white-creamy, velvety-fluffy, convex, irregularly folded, growing margin regular, undersurface brown, diameter of colony 40-45 mm. Septate branching hyphae 1-4 um wide, numerous round and oval microconidia measuring 2,2-5,0x2,5-8,0 um, single macroconidia of extended-oval shape with 1-5 cross walls, measuring 4,2-7,5x16,5-45,0 um, nonnumerous rounded arthrospores 8-10 um in diameter.
The strain was obtained by means of directed selection based on spore production and attenuation of epizootic strain.

IX. WEITERE ANGABEN ( )[8]

The strain was isolated from a camel (Kazahstan) in 1981.
It belong to the new species of dermatophyte Trichophyton sarkisovii Ivanova et Polyakov sp.nov.1983 (J.Mycologiya i phytopatologiya, Sankt-Peterburg, Russia, 1983, 17, 8,365-367).
The strain was deposited at the collection of State Scientific Control Institute of Veterinary Preparations (Moscow),No.551/63

X. HINTERLEGER[9]

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BOEHRINGER INGELHEIM VETMEDICA GBMH
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Unterschrift: L. Ivanova

Anschrift: Russia, Moscow, 115682
Zadonsky proezd, 24-1-142

Datum: 10.09.1992.

5 Ankreuzen, wenn auf einem gesonderten Blatt weitere Angaben eingereicht werden.
7 Es wird dringend empfohlen, die wissenschaftliche Beschreibung und/oder die vorgeschlagene taxonomische Bezeichnung (unter I.) des Mikroorganismus anzugeben.
8 Ankreuzen, wenn auf einem gesonderten Blatt weitere Angaben (außer den unter Fußnote 6 genannten) eingereicht werden, z. B. Herkunft des Mikroorganismus, Name und Anschrift anderer Hinterlegungsstellen, bei denen der Mikroorganismus hinterlegt worden ist, oder das für die Festlegung der vorgeschlagenen taxonomischen Bezeichnung angewandte Kriterium. (Diese Angaben sind fakultativ).
9 Der Name des Hinterlegers muß mit der Unterschrift identisch sein. Bei Firmen müssen zwei offizielle Firmenvertreter unterschreiben. Wenn natürliche Personen für eine juristische Person unterzeichnen, so ist deren Unterschrift in Maschinenschrift zu wiederholen.

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BUDAPESTER VERTRAG ÜBER DIE INTERNATIONALE ANERKENNUNG DER HINTERLEGUNG VON MIKROORGANISMEN FÜR DIE ZWECKE VON PATENTVERFAHREN

ERKLÄRUNG BEI ERSTHINTERLEGUNG
(REGEL 6.1)

An
DSM-DEUTSCHE SAMMLUNG VON
MIKROORGANISMEN UND ZELLKULTUREN GmbH
Mascheroder Weg 1b
D-3300 Braunschweig

Von der Hinterlegungsstelle auszufüllen:
DSM-Aufnahmenummer: 7279
Eingangsdatum der Kultur: Eingegangen/Received 1992-10-01 Deutsche Sammlung von Mikroorganismen

BAKTERIEN/PILZE[1]

DER UNTERZEICHNETE HINTERLEGT DEN NACHSTEHEND BEZEICHNETEN MIKROORGANISMUS AUFGRUND DES BUDAPESTER VERTRAGES UND VERPFLICHTET SICH, DIE HINTERLEGUNG WÄHREND DER IN REGEL 9.1 GENANNTEN DAUER NICHT ZURÜCKZUNEHMEN[2]

I. KENNZEICHNUNG DES MIKROORGANISMUS

Bezugszeichen[3]: No. I032
Trichophyton mentagrophytes (Robin) Blanchard, I896
Taxonomische Bezeichnung[4]:
classis Deuteromycetes
ordo Hyphomycetales
familia Mucedinaceae

Die zu hinterlegende Kultur ist:
(+) eine Reinkultur
( ) eine Mischkultur
(Zutreffendes bitte ankreuzen)

II. ZÜCHTUNGSBEDINGUNGEN ( )[5]

Medium:
beer-wort agar 7° B

pH vor der Sterilisation 7,5-7,8
Sterilisation I5 min bei I2I °C
pH nach Sterilisation: 6,3-6,9
Verhalten gegenüber Sauerstoff:
(+) aerob
( ) mikroaerophil
( ) obligat anaerob
Besondere Ansprüche an die Gasatmosphäre:

Bebrütungstemperatur: 26-28° °C
Bebrütungsdauer: I0-I5 days
Aufbewahrung bei: +?-? °C
Überimpfungsintervall: 3-4 month

[1] Die DSM nimmt zur Hinterlegung ausschließlich Mikroorganismen an, die gemäß Merkblatt 'Sichere Biotechnologie: Bakterien' (B 006 1/92 ZH 1/346) der Berufsgenossenschaft der chemischen Industrie zu den Risikogruppen 1 oder 2 gehören, bzw. unter Anwendung der Laborsicherheitsmaßnahmen L1 oder L2 entsprechend dem "Gesetz zur Regelung von Fragen der Gentechnik" (BGBL I, pp. 1080; 23/06/90) bearbeitet werden können.

[2] Dieses Formblatt kann auch verwendet werden, wenn der Unterzeichnete eine Hinterlegung eines Mikroorganismus, die er selbst oder sein Rechtsvorgänger außerhalb des Budapester Vertrags bei derselben Hinterlegungsstelle vorgenommen hat, in eine Hinterlegung nach dem Budapester Vertrag umwandelt. Dabei ist unerheblich, ob die Ersthinterlegung vor (Regel 6.4 Buchstabe d) oder nach dem Zeitpunkt stattgefunden hat, zu dem die Stelle den Status einer internationalen Hinterlegungsstelle erworben hat.

[3] Nummer, Symbole usw., die der Hinterleger dem Mikroorganismus zugeteilt hat.

[4] Es wird dringend empfohlen, die taxonomische Bezeichnung und/oder die wissenschaftliche Beschreibung des Mikroorganismus (siehe unter VII.) anzugeben.

[5] Ankreuzen, wenn auf einem besonderen Blatt weitere Angaben eingereicht werden.

The strain is stored in liophylized condition in ampulles under vacuum or on agar nutrient medium in tubes at +2-8°C.

IV. BEDINGUNGEN FÜR DIE LEBENSFÄHIGKEITSPRÜFUNG ( )[5]

With sterile pipette 3 cc of sterile phisiological solution are added to liophylised fungal matter for full resuspension of culture. Fungal suspension is seeded on sloped agar and it is cultivated during I0-I5 days at +26-28°C.
The culture from a tube is seeded on new sloped agar by spore suspension and it is cultivated during I0-I5 days at +26-28°C.

V. BESTANDTEILE DER MISCHKULTUR (wenn zutreffend) ( )[5]

Beschreibung der Bestandteile:

Verfahren, die die Prüfung auf Lebensfähigkeit der Bestandteile ermöglichen:

VI. EIGENSCHAFTEN, DIE FÜR DIE GESUNDHEIT ODER DIE UMWELT GEFÄHRLICH SIND ( )[5]

Risikogruppe des unter I. genannten Mikroorganismus gemäß Merkblatt 'Sichere Biotechnologie: Bakterien' (B 006 1/92 ZH/346) der Berufsgenossenschaft der chemischen Industrie[1]:

.....

Der zu hinterlegende Mikroorganismus darf unter folgenden Laborsicherheitsmaßnahmen[1] gehandhabt werden:

( ) L1 ( ) L2

Der Mikroorganismus hat folgende Eigenschaften, die für die Gesundheit oder die Umwelt gefährlich sind oder sein können:

Bitte angeben: The strain is weak-virulent. The result 9-I0 days after application of a dose of 500-600 thousand cells of fungal matter per cm^2 to the scarified skin of a rabbit necrotic scabs are formed.

( ) Dem Unterzeichneten sind derartige Eigenschaften nicht bekannt.

[1] Die DSM nimmt zur Hinterlegung ausschließlich Mikroorganismen an, die gemäß Merkblatt 'Sichere Biotechnologie: Bakterien' (B 006 1/92 ZH 1/346) der Berufsgenossenschaft der chemischen Industrie zu den Risikogruppen 1 oder 2 gehören, bzw. unter Anwendung der Laborsicherheitsmaßnahmen L1 oder L2 entsprechend dem "Gesetz zur Regelung von Fragen der Gentechnik" (BGBL I, pp. 1080; 23/06/90) bearbeitet werden können.

[5] Ankreuzen, wenn auf einem gesonderten Blatt weitere Angaben eingereicht werden.

VIII. WISSENSCHAFTLICHE BESCHREIBUNG[7] [5]

Mature I0-I5 day colony is cream, velvety-powdered, flat with slight flat elevation in centre, growing margin strait, fringed, undersurface light-brown, diameter of colony 25-30 mm. Septate branching hyphae I-3 um wide, numerous pyriform, oval microconidia measuring I-3x2-6 um, no macroconidia. The strain was obtained by means of directed selection based on spore production and and attenuation of epizootic strain.

IX. WEITERE ANGABEN ( )[8]

The strain was isolated from a horse in I986 (Russia). The strain was deposited at the Collection of Pathogenic Fungi within the Russian Ministry of Health Centre for Deep Mycoses in Sankt-Peterburg, No.VKPG F-930/I032.

X. HINTERLEGER[9]

Name: Dr. L.G.Ivanova Unterschrift:

Dr. I.D.Polyakov

BOEHRINGER INGELHEIM VETMEDICA GMBH

ppa. D. Janott ppa. Dr. E. Richter

Anschrift: Russia, Moscow, II5682 Datum: I0.09.I992.

Zadonsky proezd, 24-I-I42

[5] Ankreuzen, wenn auf einem gesonderten Blatt weitere Angaben eingereicht werden.

[7] Es wird dringend empfohlen, die wissenschaftliche Beschreibung und/oder die vorgeschlagene taxonomische Bezeichnung (unter I.) des Mikroorganismus anzugeben.

[8] Ankreuzen, wenn auf einem gesonderten Blatt weitere Angaben (außer den unter Fußnote 5 genannten) eingereicht werden, z. B. Herkunft des Mikroorganismus, Name und Anschrift anderer Hinterlegungsstellen, bei denen der Mikroorganismus hinterlegt worden ist, oder das für die Festlegung der vorgeschlagenen taxonomischen Bezeichnung angewandte Kriterium. (Diese Angaben sind fakultativ).

[9] Der Name des Hinterlegers muß mit der Unterschrift identisch sein. Bei Firmen müssen zwei offizielle Firmenvertreter unterschreiben. Wenn natürliche Personen für eine juristische Person unterzeichnen, so ist deren Unterschrift in Maschinenschrift zu wiederholen.

ANERKENNUNG DER HINTERLEGUNG VON MIKROORGANISMEN

FÜR DIE ZWECKE VON PATENTVERFAHREN

ERKLÄRUNG BEI ERSTHINTERLEGUNG
(REGEL 6.1)

An
DSM-DEUTSCHE SAMMLUNG VON
MIKROORGANISMEN UND ZELLKULTUREN GmbH
Mascheroder Weg 1b
D-3300 Braunschweig

Von der Hinterlegungsstelle auszufüllen:
DSM-Aufnahmenummer: 7280
Eingangsdatum der Kultur: Eingegangen/Received 1992-10-01 Deutsche Sammlung von Mikroorganismen

BAKTERIEN/PILZE[1]

DER UNTERZEICHNETE HINTERLEGT DEN NACHSTEHEND BEZEICHNETEN MIKROORGANISMUS AUFGRUND DES BUDAPESTER VERTRAGES UND VERPFLICHTET SICH, DIE HINTERLEGUNG WÄHREND DER IN REGEL 9.1 GENANNTEN DAUER NICHT ZURÜCKZUNEHMEN[2]

I. KENNZEICHNUNG DES MIKROORGANISMUS

Bezugszeichen[3]: No. I3II
Microsporum canis var.obesum (Conant) Ivanova et Polyakov, I99I
Taxonomische Bezeichnung[4]:
classis Deuteromycetes
ordo Hyphomycetales
familia Mucedinaceae

Die zu hinterlegende Kultur ist:
(+) eine Reinkultur
( ) eine Mischkultur
(Zutreffendes bitte ankreuzen)

II. ZUCHTUNGSBEDINGUNGEN ( )[5]

Medium:
beer-wort agar 7°B

pH vor der Sterilisation 7,5-7,8
Sterilisation I5min bei I2I °c
pH nach Sterilisation: 6,3-6,9
Verhalten gegenüber Sauerstoff:
(+) aerob
( ) mikroaerophil
( ) obligat anaerob
Besondere Ansprüche an die Gasatmosphäre:

Bebrütungstemperatur: 26-28 °c
Bebrütungsdauer: I0-I5 days
Aufbewahrung bei: +2-8 °c
Überimpfungsintervall: 3-4 month

1 Die DSM nimmt zur Hinterlegung ausschließlich Mikroorganismen an, die gemäß Merkblatt 'Sichere Biotechnologie: Bakterien' (B 006 1/92 ZH 1/346) der Berufsgenossenschaft der chemischen Industrie zu den Risikogruppen 1 oder 2 gehören, bzw. unter Anwendung der Laborsicherheitsmaßnahmen L1 oder L2 entsprechend dem "Gesetz zur Regelung von Fragen der Gentechnik" (BGBL I, pp. 1080; 23/06/90) bearbeitet werden können.

2 Dieses Formblatt kann auch verwendet werden, wenn der Unterzeichnete eine Hinterlegung eines Mikroorganismus, die er selbst oder sein Rechtsvorganger außerhalb des Budapester Vertrags bei derselben Hinterlegungsstelle vorgenommen hat, in eine Hinterlegung nach dem Budapester Vertrag umwandelt. Dabei ist unerheblich, ob die Ersthinterlegung vor (Regel 6.4 Buchstabe d) oder nach dem Zeitpunkt stattgefunden hat, zu dem die Stelle den Status einer internationalen Hinterlegungsstelle erworben hat.

3 Nummer, Symbole usw., die der Hinterleger dem Mikroorganismus zugeteilt hat.

4 Es wird dringend empfohlen, die taxonomische Bezeichnung und/oder die wissenschaftliche Beschreibung des Mikroorganismus (siehe unter VII.) anzugeben.

5 Ankreuzen, wenn auf einem besonderen Blatt weitere Angaben eingereicht werden.

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III. AUFBEWAHRUNGSBEDINGUNGEN ( )[5]

The strain is stored in liophylized condition in ampulles under vacuum or on agar nutrient medium in tubes at +2-8°C.

IV. BEDINGUNGEN FÜR DIE LEBENSFÄHIGKEITSPRÜFUNG ( )[5]

With sterile pipette 2 cc of sterile phisiological solution are added to liophylised fungal material for full resuspension of culture. Fungal suspension is seeded on sloped agar and it is cultivated during 15-20 days at +26-28°C.
The culture from a tube is seeded on new sloped agar by spore suspension and it is cultivated during 10-15 days at +26-28°C.

V. BESTANDTEILE DER MISCHKULTUR (wenn zutreffend) ( )[5]

Beschreibung der Bestandteile:

Verfahren, die die Prüfung auf Lebensfähigkeit der Bestandteile ermöglichen:

VI. EIGENSCHAFTEN, DIE FÜR DIE GESUNDHEIT ODER DIE UMWELT GEFÄHRLICH SIND ( )[5]

Risikogruppe des unter I. genannten Mikroorganismus gemäß Merkblatt 'Sichere Biotechnologie: Bakterien' (B 006 1/92 ZH/346) der Berufsgenossenschaft der chemischen Industrie[1]:

.....

Der zu hinterlegende Mikroorganismus darf unter folgenden Laborsicherheitsmaßnahmen[1] gehandhabt werden:

( ) L1 ( ) L2

Der Mikroorganismus hat folgende Eigenschaften, die für die Gesundheit oder die Umwelt gefährlich sind oder sein können:

Bitte angeben: The strain is weak-virulent. The result 9-11 days after application of a dose of 500-600 thousand cells of fungal matter per cm^2 to the scarified skin of a rabbit necrotic scabs are formed. Spontaneous recovery following 22-25 days.

( ) Dem Unterzeichneten sind derartige Eigenschaften nicht bekannt.

[1] Die DSM nimmt zur Hinterlegung ausschließlich Mikroorganismen an, die gemäß Merkblatt 'Sichere Biotechnologie: Bakterien' (B 006 1/92 ZH 1/346) der Berufsgenossenschaft der chemischen Industrie zu den Risikogruppen 1 oder 2 gehören, bzw. unter Anwendung der Laborsicherheitsmaßnahmen L1 oder L2 entsprechend dem "Gesetz zur Regelung von Fragen der Gentechnik" (BGBL I, pp. 1080; 23/06/90) bearbeitet werden können.

[5] Ankreuzen, wenn auf einem gesonderten Blatt weitere Angaben eingereicht werden.

VIII. WISSENSCHAFTLICHE BESCHREIBUNG[7] ( )[5]

Mature 10-15 day colony is white, fluffy, flat with a denser central dome-like elevation, growing margin strait, fringed, undersurface colourless with brown centre, diameter of colony 30-35 mm. Septate branching hyphae 1-3 um wide, numerous pyriform, oval and cylindrical microconidia measuring 1-3x3-7 um, few short, elliptical, fusiform, stretched-oval macroconidia, some irregular shapes, less frequently "beaked", with 2-5 septates, measuring 11-20x25-50 um.

IX. WEITERE ANGABEN ( )[8]

The strain was obtained by means of directed selection based on spore production and attenuation of epizootic strain. The strain was isolated from a tiger in 1986 (Russia). It belong to the new combination of dermatophyte Microsporum canis var.obesum Ivanova et Polyakov comb.nov.,1991 (Dissertation). It was deposited at the Collection of Pathogenic Fungi in Sankt-Petersburg (Russia), No.VKPG F-727/1311.

X. HINTERLEGER[9]

Name: Dr. L.G.Ivanova
Dr. I.D.Polyakov
BOEHRINGER INGELHEIM VETMEDICA GMBH
pda.
D. Janott Dr. E. Richter

Unterschrift:

Anschrift: Russia, Moscow 115682
Zadonsky proezd, 24-1-142

Datum: 10.09.1992.

5 Ankreuzen, wenn auf einem gesonderten Blatt weitere Angaben eingereicht werden.
7 Es wird dringend empfohlen, die wissenschaftliche Beschreibung und/oder die vorgeschlagene taxonomische Bezeichnung (unter I.) des Mikroorganismus anzugeben.
8 Ankreuzen, wenn auf einem gesonderten Blatt weitere Angaben (außer den unter Fußnote 5 genannten) eingereicht werden, z. B. Herkunft des Mikroorganismus, Name und Anschrift anderer Hinterlegungsstellen, bei denen der Mikroorganismus hinterlegt worden ist, oder das für die Festlegung der vorgeschlagenen taxonomischen Bezeichnung angewandte Kriterium. (Diese Angaben sind fakultativ).
9 Der Name des Hinterlegers muß mit der Unterschrift identisch sein. Bei Firmen müssen zwei offizielle Firmenvertreter unterschreiben. Wenn natürliche Personen für eine juristische Person unterzeichnen, so ist deren Unterschrift in Maschinenschrift zu wiederholen.

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ANERKENNUNG DER HINTERLEGUNG VON MIKROORGANISMEN

FÜR DIE ZWECKE VON PATENTVERFAHREN

ERKLÄRUNG BEI ERSTHINTERLEGUNG
(REGEL 6.1)

An
DSM-DEUTSCHE SAMMLUNG VON
MIKROORGANISMEN UND ZELLKULTUREN GmbH
Mascheroder Weg 1b
D-3300 Braunschweig

Von der Hinterlegungsstelle auszufüllen:
DSM-Aufnahmenummer: 7281
Eingangsdatum der Kultur: Eingegangen/Received 1992-10-01 Deutsche Sammlung von Mikroorganismen

BAKTERIEN/PILZE[1]

DER UNTERZEICHNETE HINTERLEGT DEN NACHSTEHEND BEZEICHNETEN MIKROORGANISMUS AUFGRUND DES BUDAPESTER VERTRAGES UND VERPFLICHTET SICH, DIE HINTERLEGUNG WÄHREND DER IN REGEL 9.1 GENANNTEN DAUER NICHT ZURÜCKZUNEHMEN[2]

I. KENNZEICHNUNG DES MIKROORGANISMUS

Bezugszeichen[3]: No. I393
Microsporum canis Bodin, I902

Taxonomische Bezeichnung[4]:
classis Deuteromycetes
ordo Hyphomycetales
familia Mucedinaceae

Die zu hinterlegende Kultur ist:
(+) eine Reinkultur
( ) eine Mischkultur
(Zutreffendes bitte ankreuzen)

II. ZÜCHTUNGSBEDINGUNGEN ( )[5]

Medium:
beer-wort agar 7°B

pH vor der Sterilisation 7,5-7,8
Sterilisation I5 min bei I2I °c
pH nach Sterilisation: 6,3-6;9
Verhalten gegenüber Sauerstoff:
(+) aerob
( ) mikroaerophil
( ) obligat anaerob
Besondere Ansprüche an die Gasatmosphäre:

Bebrütungstemperatur: 26-28 °c
Bebrütungsdauer: I0-I5 days
Aufbewahrung bei: +2-8 °c
Überimpfungsintervall: 3-4 month

1 Die DSM nimmt zur Hinterlegung ausschließlich Mikroorganismen an, die gemäß Merkblatt 'Sichere Biotechnologie: Bakterien' (B 006 1/92 ZH 1/346) der Berufsgenossenschaft der chemischen Industrie zu den Risikogruppen 1 oder 2 gehören, bzw. unter Anwendung der Laborsicherheitsmaßnahmen L1 oder L2 entsprechend dem "Gesetz zur Regelung von Fragen der Gentechnik" (BGBL I, pp. 1080; 23/06/90) bearbeitet werden können.

2 Dieses Formblatt kann auch verwendet werden, wenn der Unterzeichnete eine Hinterlegung eines Mikroorganismus, die er selbst oder sein Rechtsvorgänger außerhalb des Budapester Vertrags bei derselben Hinterlegungsstelle vorgenommen hat, in eine Hinterlegung nach dem Budapester Vertrag umwandelt. Dabei ist unerheblich, ob die Ersthinterlegung vor (Regel 6.4 Buchstabe d) oder nach dem Zeitpunkt stattgefunden hat, zu dem die Stelle den Status einer internationalen Hinterlegungsstelle erworben hat.

3 Nummer, Symbole usw., die der Hinterleger dem Mikroorganismus zugeteilt hat.

4 Es wird dringend empfohlen, die taxonomische Bezeichnung und/oder die wissenschaftliche Beschreibung des Mikroorganismus (siehe unter VII.) anzugeben.

5 Ankreuzen, wenn auf einem besonderen Blatt weitere Angaben eingereicht werden.

III. AUFBEWAHRUNGSBEDINGUNGEN ( )[5]

The strain is stored in liophylized condition in ampulles under vacuum or on agar nutrient medium in tubes at +2-8°C.

IV. BEDINGUNGEN FÜR DIE LEBENSFÄHIGKEITSPRÜFUNG ( )[5]

With sterile pipette 3 cc sterile phisiological solution are added to liophylized fungal matter for full resuspension of culture. Fungal suspension is seeded on sloped agar and it is cultivated during 10-15 days at +26-28°C.
The culture from a tube is seeded on new sloped agar by spore suspension and it is cultivated during 10-15 days at +26-28°C.

V. BESTANDTEILE DER MISCHKULTUR (wenn zutreffend) ( )[5]

Beschreibung der Bestandteile:

Verfahren, die die Prüfung auf Lebensfähigkeit der Bestandteile ermöglichen:

VI. EIGENSCHAFTEN, DIE FÜR DIE GESUNDHEIT ODER DIE UMWELT GEFÄHRLICH SIND ( )[5]

Risikogruppe des unter I. genannten Mikroorganismus gemäß Merkblatt 'Sichere Biotechnologie: Bakterien' (B 006 1/92 ZH/346) der Berufsgenossenschaft der chemischen Industrie[1]:

.....

Der zu hinterlegende Mikroorganismus darf unter folgenden Laborsicherheitsmaßnahmen[1] gehandhabt werden:

( ) L1 ( ) L2

Der Mikroorganismus hat folgende Eigenschaften, die für die Gesundheit oder die Umwelt gefährlich sind oder sein können:

Bitte angeben: The strain is weak-virulent. The result 9-11 days after application of a dose of 500-600 thousand cells of fungal matter per cm^2 to the scarified skin of a rabbit necrotic scabs are formed. Spontaneous recovery following 20-24 days.

( ) Dem Unterzeichneten sind derartige Eigenschaften nicht bekannt.

[1] Die DSM nimmt zur Hinterlegung ausschließlich Mikroorganismen an, die gemäß Merkblatt 'Sichere Biotechnologie: Bakterien' (B 006 1/92 ZH 1/346) der Berufsgenossenschaft der chemischen Industrie zu den Risikogruppen 1 oder 2 gehören, bzw. unter Anwendung der Laborsicherheitsmaßnahmen L1 oder L2 entsprechend dem "Gesetz zur Regelung von Fragen der Gentechnik" (BGBL I, pp. 1080; 23/06/90) bearbeitet werden können.

[5] Ankreuzen, wenn auf einem gesonderten Blatt weitere Angaben eingereicht werden.

VIII. WISSENSCHAFTLICHE BESCHREIBUNG[7] ( )[5]

Mature 10-15 day colony is white, fluffy, convex, growing margin strait, arachnoid, undersurface brown, diameter of colony 30-35 mm. Septate branching hyphae 1-4 um wide, numerous pyriform, cylindrical microconidia measuring 1-3x3-7 um, few fusiform macroconidia with 3-11 septates, measuring 10-20x40-75 um.
The strain was obtained by means of directed selection based on spore production and attenuation of epizootic strain.

IX. WEITERE ANGABEN ( )[8]

The strain was isolated from a cat in 1988 (Russia).
The strain was deposited at the Collection of Pathogenic Fungi within the Russian Ministry of Health Centre for Deep Mycoses in Sankt-Peterburg, No.VKPG F-928/1393.

X. HINTERLEGER[9]

Name: Dr. L.G.Ivanova
Dr. I.D.Polyakov
BOEHRINGER INGELHEIM VETMEDICA GMBH
ppa.
Dr. F. Richter

Unterschrift:

Anschrift: D. Janott
Russia, Moscow 115682
Zadonsky proezd, 24-1-142

Datum: 10.09.1992.

5 Ankreuzen, wenn auf einem gesonderten Blatt weitere Angaben eingereicht werden.
7 Es wird dringend empfohlen, die wissenschaftliche Beschreibung und/oder die vorgeschlagene taxonomische Bezeichnung (unter I.) des Mikroorganismus anzugeben.
8 Ankreuzen, wenn auf einem gesonderten Blatt weitere Angaben (außer den unter Fußnote 5 genannten) eingereicht werden, z. B. Herkunft des Mikroorganismus, Name und Anschrift anderer Hinterlegungsstellen, bei denen der Mikroorganismus hinterlegt worden ist, oder das für die Festlegung der vorgeschlagenen taxonomischen Bezeichnung angewandte Kriterium. (Diese Angaben sind fakultativ).
9 Der Name des Hinterlegers muß mit der Unterschrift identisch sein. Bei Firmen müssen zwei offizielle Firmenvertreter unterschreiben. Wenn natürliche Personen für eine juristische Person unterzeichnen, so ist deren Unterschrift in Maschinenschrift zu wiederholen.

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BUDAPESTER VERTRAG ÜBER DIE INTERNATIONALE ANERKENNUNG DER HINTERLEGUNG VON MIKROORGANISMEN FÜR DIE ZWECKE VON PATENTVERFAHREN

INTERNATIONALES FORMBLATT

Boehringer Ingelheim
Vetmedica GmbH
Binger Str. 173

55216 Ingelheim

EMPFANGSBESTÄTIGUNG BEI ERSTHINTERLEGUNG, ausgestellt gemäß Regel 7.1 von der unten angegebenen INTERNATIONALEN HINTERLEGUNGSSTELLE

I. KENNZEICHNUNG DES MIKROORGANISMUS	
Vom HINTERLEGER zugeteiltes Bezugszeichen: No. 008	Von der INTERNATIONALEN HINTERLEGUNGSSTELLE zugeteilte EINGANGSNUMMER: DSM 9656

II. WISSENSCHAFTLICHE BESCHREIBUNG UND/ODER VORGESCHLAGENE TAXONOMISCHE BEZEICHNUNG

Mit dem unter I. bezeichneten Mikroorganismus wurde

(X) eine wissenschaftliche Beschreibung
(X) eine vorgeschlagene taxonomische Bezeichnung

eingereicht.
(Zutreffendes ankreuzen).

III. EINGANG UND ANNAHME

Diese internationale Hinterlegungsstelle nimmt den unter I bezeichneten Mikroorganismus an, der bei ihr am 1994-12-28 (Datum der Ersthinterlegung)[1] eingegangen ist.

IV. EINGANG DES ANTRAGS AUF UMWANDLUNG

Der unter I bezeichnete Mikroorganismus ist bei dieser Internationalen Hinterlegungsstelle am eingegangen (Datum der Ersthinterlegung) und ein Antrag auf Umwandlung dieser Ersthinterlegung in eine Hinterlegung gemäß Budapester Vertrag ist am eingegangen (Datum des Eingangs des Antrags auf Umwandlung).

V. INTERNATIONALE HINTERLEGUNGSSTELLE

Name: DSM-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Anschrift: Mascheroder Weg 1b D-38124 Braunschweig	Unterschrift(en) der zur Vertretung der internationalen Hinterlegungsstelle befugten Person(en) oder des (der) von ihr ermächtigten Bediensteten: U Weiss Datum: 1995-01-09

[1] Falls Regel 6.4 Buchstabe d zutrifft, ist dies der Zeitpunkt, zu dem der Status einer internationalen Hinterlegungsstelle erworben worden ist.

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BUDAPEST TREATY ON THE INTERNATIONAL

RECOGNITION OF THE DEPOSIT OF MICROORGANISMS

FOR THE PURPOSES OF PATENT PROCEDURE

STATEMENT IN THE CASE OF AN ORIGINAL DEPOSIT
pursuant to Rule 6.1

To
DSM-DEUTSCHE SAMMLUNG VON
MIKROORGANISMEN UND ZELLKULTUREN GmbH
Mascheroder Weg 1b
D-38124 Braunschweig
Federal Republic of Germany

To be filled in by the Depositary Authority

DSM-Accession Number :

Date culture received:

BACTERIA/FUNGI[1]

THE UNDERSIGNED HEREBY DEPOSITS UNDER THE BUDAPEST TREATY THE MICROORGANISM IDENTIFIED HEREUNDER AND UNDERTAKES NOT TO WITHDRAW THE DEPOSIT FOR THE PERIOD SPECIFIED IN RULE 9.1[2]

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference[3] : No. 008
Candida albicans
(Robin 1853) Berkhout 1923

Taxonomic designation[4] :
classis Deuteromycetes
ordo Hyphomycetales
familia Cryptococcaceae

The culture to be deposited is :

(x) a pure culture

( ) a mixture of microorganisms

(Mark with a cross where applicable)

II. CONDITIONS FOR CULTIVATION ( )[5]

Medium:

agar Sabouraud

pH before sterilisation: 6,3 - 6,5

Sterilisation 15 min at 121° ·c

pH after sterilisation: 6,0 - 6,4

Oxygen relationship:

(x) aerobic

( ) microaerophilic

( ) obligate anaerobic

Specific gaseous requirements:

Incubation temperature: 37° ·c

Incubation time: 2 days

Short term storage at: + 2 - 8°·c

Interval of transfer: 2 - 3 months

1 The DSM only accepts for deposit microorganisms which belong to hazard group 1 or 2, according to the BG-Chemie leaflet 'Safe Biotechnology', Classification of biological agents: Bacteria (B 006e), Fungi (B 007e) and can be classified as S1 or S2 organisms according to "Gesetz zur Regelung von Fragen der Gentechnik" (BGBL I, pp. 1080; 23/06/90).

2 This form may also be used if the undersigned converts into a deposit under the Budapest Treaty the deposit of a microorganism that he or his predecessor in title has already deposited, outside the Budapest Treaty, with the same depositary institution either before (Rule 6.4(d)) or after the acquisition by that institution of the status of international depositary authority.

3 Number, symbols etc., given to the microorganism by the depositor.

4 It is strongly recommended to indicate the taxonomic designation and/or scientific description (see under VII.) of the microorganism.

5 Mark with a cross if additional information is given on an attached sheet.

III. CONDITIONS FOR LONG TERM STORAGE ( )[5]

The strain is kept on agar nutrient medium in tubes at + 2 - 8° C.

IV. CONDITIONS FOR TESTING VIABILITY ( )[5]

The fungal cells from a tube are seeded on a new agar slope and are cultivated for 2 days at 37° C.

V. COMPONENTS OF MIXED CULTURE (WHEN APPLICABLE) ( )[5]

Description of components:

Method(s) for checking presence of components:

VI. PROPERTIES DANGEROUS TO HEALTH OR ENVIRONMENT ( )[5]

Hazard group of the microorganism according to 'Safe Biotechnology, Classification of biological agents: Bacteria (B 006e), Fungi (B 007e):[1]

THE STRAIN HAS TO BE HANDLED UNDER THE FOLLOWING LABORATORY CONTAINMENT LEVEL[1]:

( ) L1 ( ) L2

IS THIS STRAIN DANGEROUS TO HEALTH OR THE ENVIRONMENT ? (x) YES ( ) NO

if yes, please specify:

The strain is weakly virulent. 30 days after intraperitoneal injection by dose of 10 - 100 mio fungal cells to white mice granuloma in abdominal organs at 80 % of animals are formed. Lethal effect doesn't observe.

( ) the undersigned is not aware of such properties

[1] The DSM only accepts for deposit microorganisms which belong to hazard group 1 or 2, according to the BG-Chemie leaflet 'Safe Biotechnology', Classification of biological agents: Bacteria (B 006e), Fungi (B 007e) and can be classified as S1 or S2 organisms according to "Gesetz zur Regelung von Fragen der Gentechnik" (BGBL I, pp. 1080; 23/06/90).

[5] Mark with a cross if additional information is given on an attached sheet

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VII. IF THE MICROORGANISM IS GENETICALLY MANIPULATED not applicable ( )[5]
Complete answers to be given!

1. DATA CONCERNING THE HOST ORGANISM

designation:

hazard group: ( ) haz. gr. 1 ( ) haz. gr. 2
biological safety grade: ( ) B1 ( ) B2

sensitivities:
resistances:

auxotrophies:

special properties:
(e.g restriction/modification system, general genetic recombination)

2. DATA CONCERNING THE DONOR ORGANISM

designation:

hazard group: ( ) haz. gr. 1 ( ) haz. gr. 2 ( ) haz. gr. 3

description of the cloned DNA fragment:
cloned information:

size of the cloned DNA: ( ) complete genome ( ) cDNA
(in bp) ( ) subgenomic
( ) subgenic

potential risk of the cloned DNA:

( ) no potential risk ( ) pathogenic ( ) tumorigenic
( ) toxigenic ( ) allergenic

3. DATA CONCERNING THE VECTOR

designation:

derivative of:
biological safety grade: ( ) B1 ( ) B2

host specificity:

resistances:

plasmid/virus size:

promoters:

additional reading frames:

own infectiosity: ( ) yes ( ) no
mobilisable plasmid: ( ) yes ( ) no
own transfer system: ( ) yes ( ) no
transfer by endogenous helper viruses: ( ) yes ( ) no

4. DATA CONCERNING THE GENETICALLY MANIPULATED ORGANISM

special properties:
(e.g. production of ...; use as ...-vector etc.)

foreign DNA: ( ) chromosomally integrated ( ) episomal

potential risk: ( ) pathogenic ( ) tumorigenic
( ) toxigenic ( ) allergenic

( ) no potential risk
please indicate why:

According to the regulations of the GenTG[6] the DSM can only accept genetically manipulated, potentially pathogenic organisms for deposition when a copy of the permit issued by the competent authority (or by an equivalent national biological safety commission) for work on the organisms accompanies the deposition form.

5 Mark with a cross if additional information is given on an attached sheet.
6 GenTG = Gesetz zur Regelung von Fragen der Gentechnik (German law for the regulation of questions concerning genetic engineering)

VIII. SCIENTIFIC DESCRIPTION[7] ( )[6]

10-day colony on agar Sabouraud is cream smooth pasty glistening, elevated, with central depression, margin of colony regular, diameter of colony 18-22 mm. Spherical oval blastospores measuring 3,5-5x5-8 mkm, pseudohypha 5-8 mkm wide, hypha 2-3 mkm wide.
Chlamidospores on rise agar 13-16 mkm in diameter.

IX. ADDITIONAL DATA ( )[8]

The strain was isolated from man in 1990.
The strain was obtained by means of directed selection based on stabilisation of cultural-morphological characteristics and attenuation of epidemic strain.

X. DEPOSITOR[9]

Please note that the depositor must be identical with the patent applicant.

Name:
BOEHRINGER INGELHEIM VETMEDICA GMBH

Address:
Binger Str. 173
55216 Ingelheim

Signature:
ppa: Dr. Laudien

i. V.: Dr. Hoffmann

Date:
23.12.1994

[5] Mark with a cross if additional information is given on an attached sheet.
[7] It is strongly recommended to indicate the scientific description and/or proposed taxonomic designation (see I.) of the microorganism.
[8] Mark with a cross if additional information (other than the information referred to in footnote 5 is given on an attached sheet, such as the source of the microorganism, the name(s) and the address(es) of any other depositary institution(s) with which the microorganism has been deposited, or the criterion used when drafting the proposed taxonomic designation (The supplying of such information is optional).
[9] This Deposition Form must be signed by the depositor.
In case of a legal entity the signatures of two representatives, officially nominated by this entity, are required.
Where the signature is required on behalf of a legal entity, the typewritten name(s) of the natural person(s) signing on behalf of the legal entity should accompany the signature(s).

Claims

1. Process for the preparation of antigenic soluble material comprising polysaccharide and/or glycopeptide (ASMP), characterised in that fungal cells, which belong to the group of keratinophilic fungi or yeasts, or material thereof

- are treated under aqueous alkaline conditions,
- the solid and liquid phases of the preparation are separated,
- after separation the supernatant is treated with mineral or organic acid,
  and
- after separation ASMP is precipitated from the supernatant.

2. Process according to claim 1, characterised in that said fungal cells or material thereof

- are treated with about 0.1-5 %(w/v) KOH or NaOH, at about 20 -150 C, for up to 30h,
- are centrifuged,
- after centrifugation the supernatant is treated with 0.2 - 1.5 M organic acid or 0.05 - 1M mineral acid,
- after centrifugation the supernatant is treated with a suitable organic solvent or a salt, e.g. with an alcohol such as a lower alkanol, or ammonium sulphate, and
- the precipitate is recovered and if desired dissolved in aqueous solution.

3. Process for the preparation of antigenic non soluble material comprising polysaccharide and/or glycopeptide (ANMP), characterised in that fungal cells, belonging to the group of keratinophilic fungi or yeasts, or material thereof

- are treated under aqueous alkaline conditions,
- the solid and liquid phases of the preparation are separated, and
- after separation the solid phase is treated with mineral or organic acid.

4. Process according to claim 3, characterised in that said fungal cells or material thereof

- are treated with about 0.1-5 %(w/v) KOH or NaOH, at about 20 -150 C, for up to 30h,
- the solid phase is treated with 0.2 - 1.5 M organic acid or 0.05 - 1M mineral acid, and
- washed with an aqueous solution.

5. Process for the preparation of antigenic exogenous material comprising polysaccharide and/or glycopeptide (AEMP), characterised in that fungal cells, belonging to the group of keratinophilic fungi or yeasts, or material thereof

- are cultivated in liquid medium,
- the solid and liquid phases of the preparation are separated, and
- after separation AEMP is precipitated from the supernatant.

6. Process according to claim 5 characterised in that

- the cultivation is for up to 250h,
- after separation an alcohol is added to the supernatant, and
- the precipitate is recovered and if desired dissolved in aqueous solution.

7. Process according to claim 5 or 6 characterised in that

- after separation the supernatant is lyophilised,
- dissolved in aqueous solution,
- after precipitation with about 1-5 volumes of an alcohol the precipitate is dissolved in aqueous solution,
- the solution is lyophilised.

8. Process according to one of claims 1 to 7, characterised in that said keratinophilic fungus, belongs to at least one of the following fungi genera *Trichophyton* and/or *Microsporum* and/or said yeast belongs to the genus *Candida*.

9. Process according to one of claims 1 to 7, characterised in that said fungus belongs to any one of the following fungi species:

- *Trichophyton equinum*,
- *Trichophyton mentagrophytes*,
- *Trichophyton sarkisovii*,
- *Trichophyton verrucosum*,
- *Microsporum canis*,
- *Microsporum gypseum*, or

- *Candida albicans*.

10. Process according to one of claims 1 to 7, characterised in that said fungus belongs to any one of the following fungi strains:

- *Trichophyton equinum* DSM No. 7276,
- *Trichophyton mentagrophytes* DSM No. 7279,
- *Trichophyton sarkisovii* DSM No. 7278,
- *Trichophyton verrucosum* DSM No. 7277,
- *Microsporum canis* DSM No. 7281,
- *Microsporum canis var. obesum* DSM No. 7280,
- *Microsporum canis var. distortum* DSM No. 7275,
- *Microsporum gypseum* DSM No. 7274, or
- *Candida albicans* DSM No. 9656.

11. A polysaccharide and/or glycopeptide - comprising material having anti-allergy activity in mammals, said material being derived, or derivable, from keratinophilic fungi or yeasts or from material thereof by suitable isolation techniques, for example those defined in any of claims 1, 3 and 5.

12. Material (ASMP) preparable as defined in claim 1 or 2 or any one of claims 8 to 10.

13. Material (ANMP) preparable as defined in claim 3 or 4 or any one of claims 8 to 10.

14. Material (AEMP) preparable as defined in any one of claims 5 to 7 or any one of claims 8 to 10.

15. Material according to claim 11 or claim 12, characterised in that it contains ASMP from strain *T. mentagrophytes* DSM No. 7279, ASMP from strain *M. gypseum* DSM No. 7274 and ASMP from strain *C. albicans* DSM No. 9656.

16. -Material according to claim 11 or claim 13, characterised in that it contains ANMP from strain *T. mentagrophytes* DSM No. 7279, ANMP from strain *M. gypseum* DSM No. 7274 and ANMP from strain *C. albicans* DSM No. 9656.

17. Material according to claim 11 or claim 14, characterised in that it contains AEMP from strain *T. mentagrophytes* DSM No. 7279, AEMP from strain *M. gypseum* DSM No. 7274 and AEMP from strain *C. albicans* DSM No. 9656.

18. Material comprising any combination of the materials as defined in any one of claims 11 to 17.

19. Material according to claim 18, characterised in that it contains ASMP and AEMP.

20. Material according to claim 18, characterised in that it contains ASMP and AEMP and ANMP.

21. Material according to claim 18, characterised in that it contains AEMP and ANMP.

22. Material according to claim 18, characterised in that it contains ASMP and ANMP.

23. Vaccine comprising material as defined in any one of claims 11 to 22.

24. -Vaccine comprising material as defined in any one of claims 11 to 23 together with a suitable physiological carrier.

25. Solution for injection comprising material as defined in any one of claims 11-24.

26. Material as claimed in any one of claims 11 to 25 for pharmaceutical use.

27. Use of material as defined in any one of claims 11 to 25 as a pharmaceutical product.

28. Use of material as defined in any one of claims 11 to 25 for the preparation of a pharmaceutical product for the prophylaxis and/or treatment of allergy.

29. Use of material as defined in any one of claims 11 to 25 for the preparation of a pharmaceutical product for modulating the immune response.

30. A method for the prophylaxis and/or treatment of allergy comprising administering to a mammal an effective amount of a material as defined in any one of claims 11 to 25.

31. A method of modulating the immune response of a mammal comprising administering to a mammal an effective amount of a material as defined in any one of claims 11 to 25.

32. *Candida albicans* as deposited at the 'Deutsche Sammlung für Mikroorganismen (DSM)' under Accession No. 9656, and mutants thereof which also have low pathogenicity and which also provide material as defined in claim 11.

33. Material according to claim 11 or claim 12, characterised in that it contains ASMP from strain *M. gypseum* DSM No. 7274 and ASMP from strain *C. albicans* DSM No. 9656.

34. Use of material as defined in any one of claims 11 to 25 or 33 for the preparation of a pharmaceutical product for the prophylaxis or treatment of summer eczema.

35. Use of material as defined in any one of claims 11 to 25 or 33 for the preparation of a pharmaceutical product for the prophylaxis or treatment of alopecia.

36. Use of material as defined in any one of claims 11 to 25 or 33 for the preparation of a pharmaceutical product for the prophylaxis or treatment of eczema.

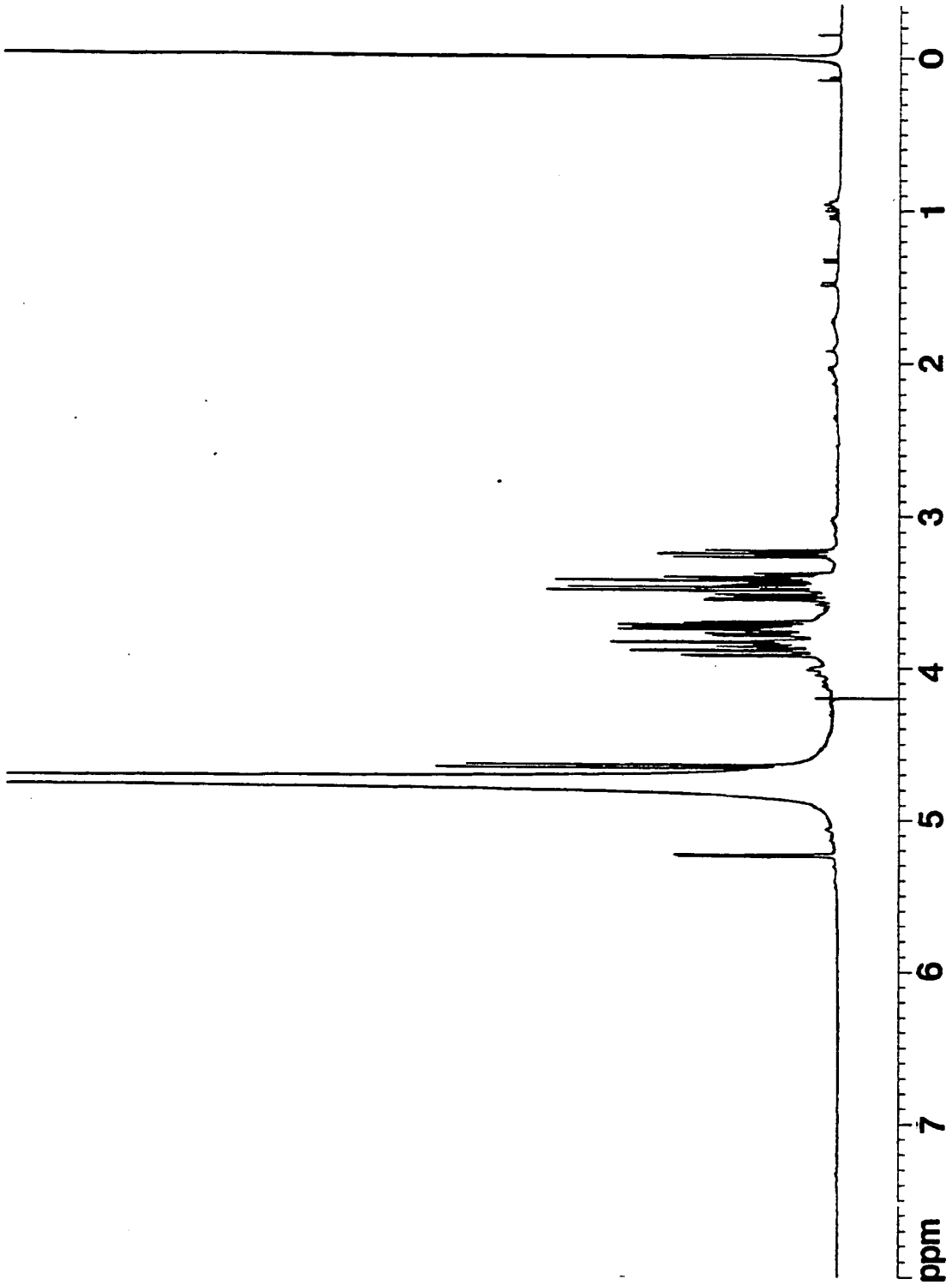
37. Use of material as defined in any one of claims 11 to 25 or 33 for the preparation of a pharmaceutical

product for the prophylaxis or treatment of neurodermitis.

38. Use of material as defined in any one of claims 11 to 25 or 33 for the preparation of a pharmaceutical product for improving the hairy coat on a mammal.

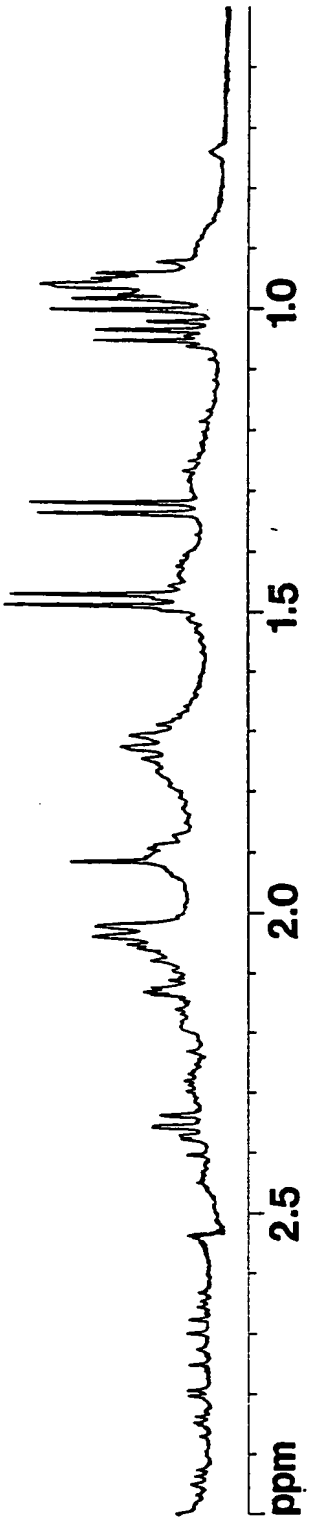
TM 7279/
p32-5-1

Fig. 1A



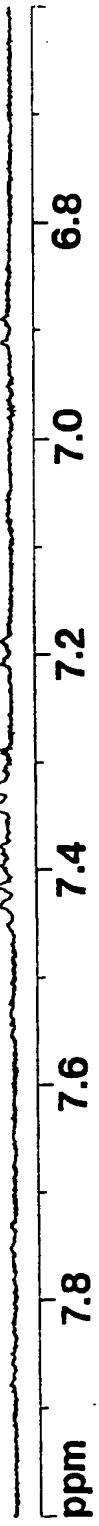
TM 7279
p32-5-1

Fig. 1B



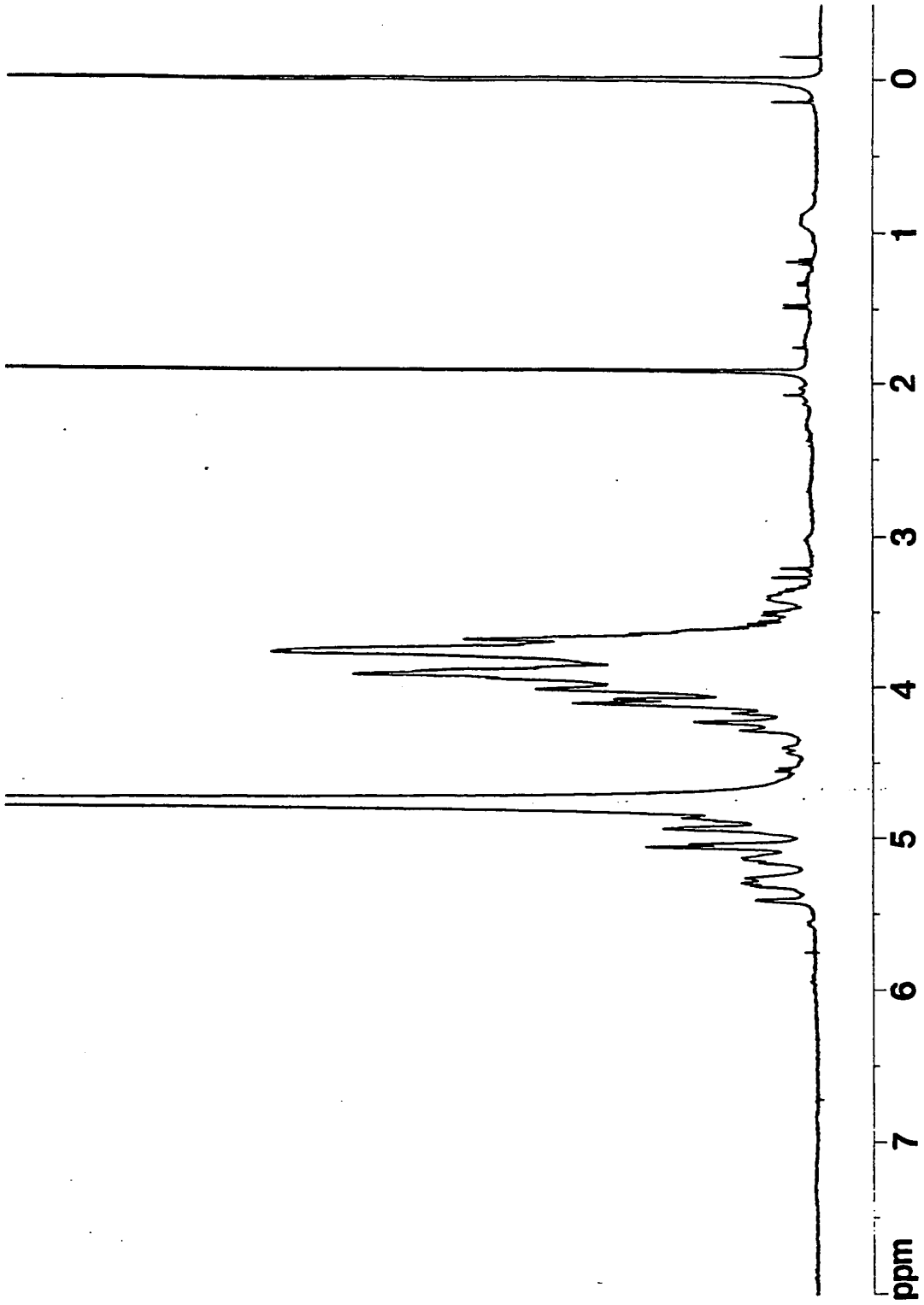
TM 7279/
p32-5-1

Fig. 1C



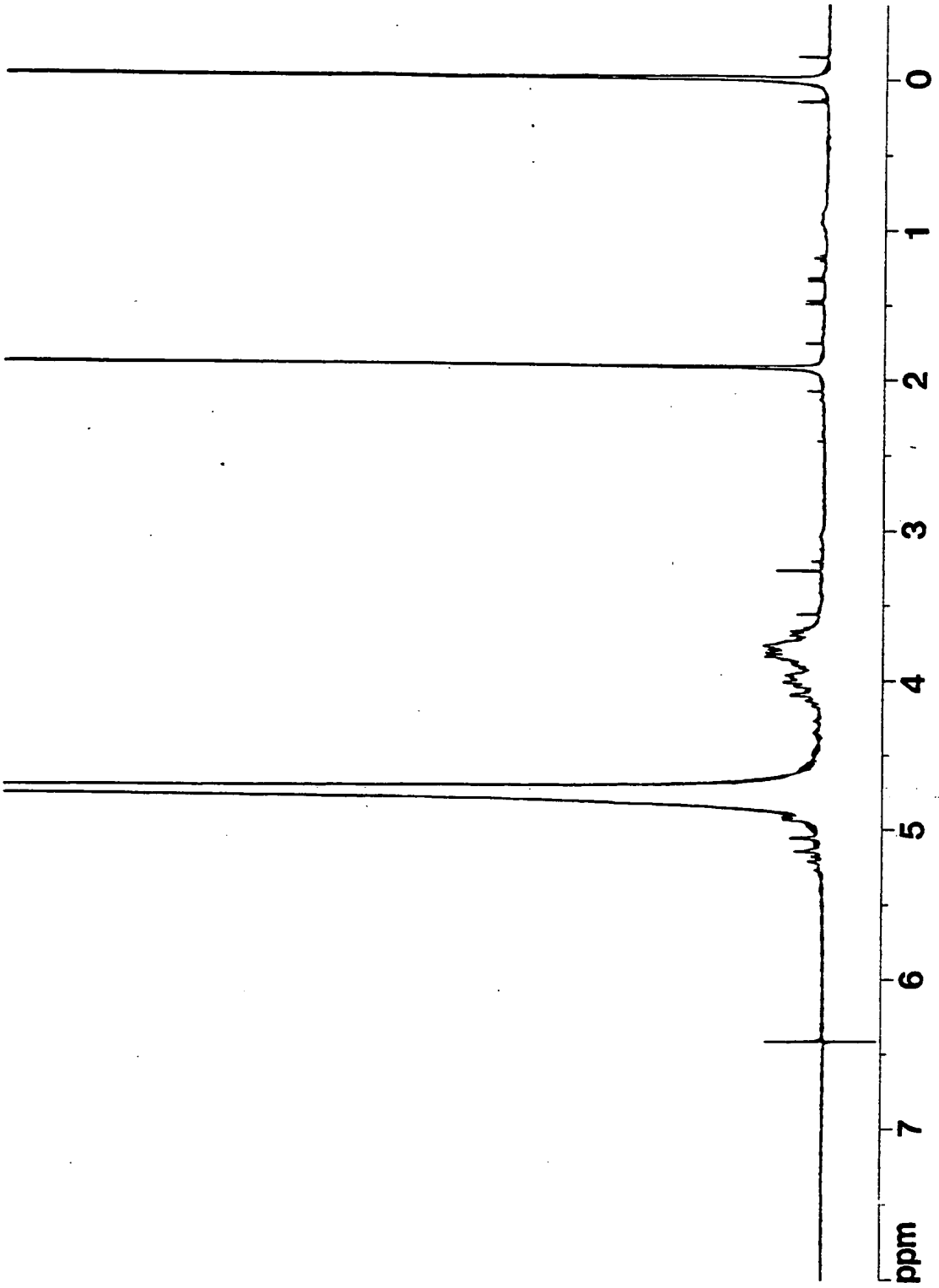
CA 9656/
b008

Fig. 2



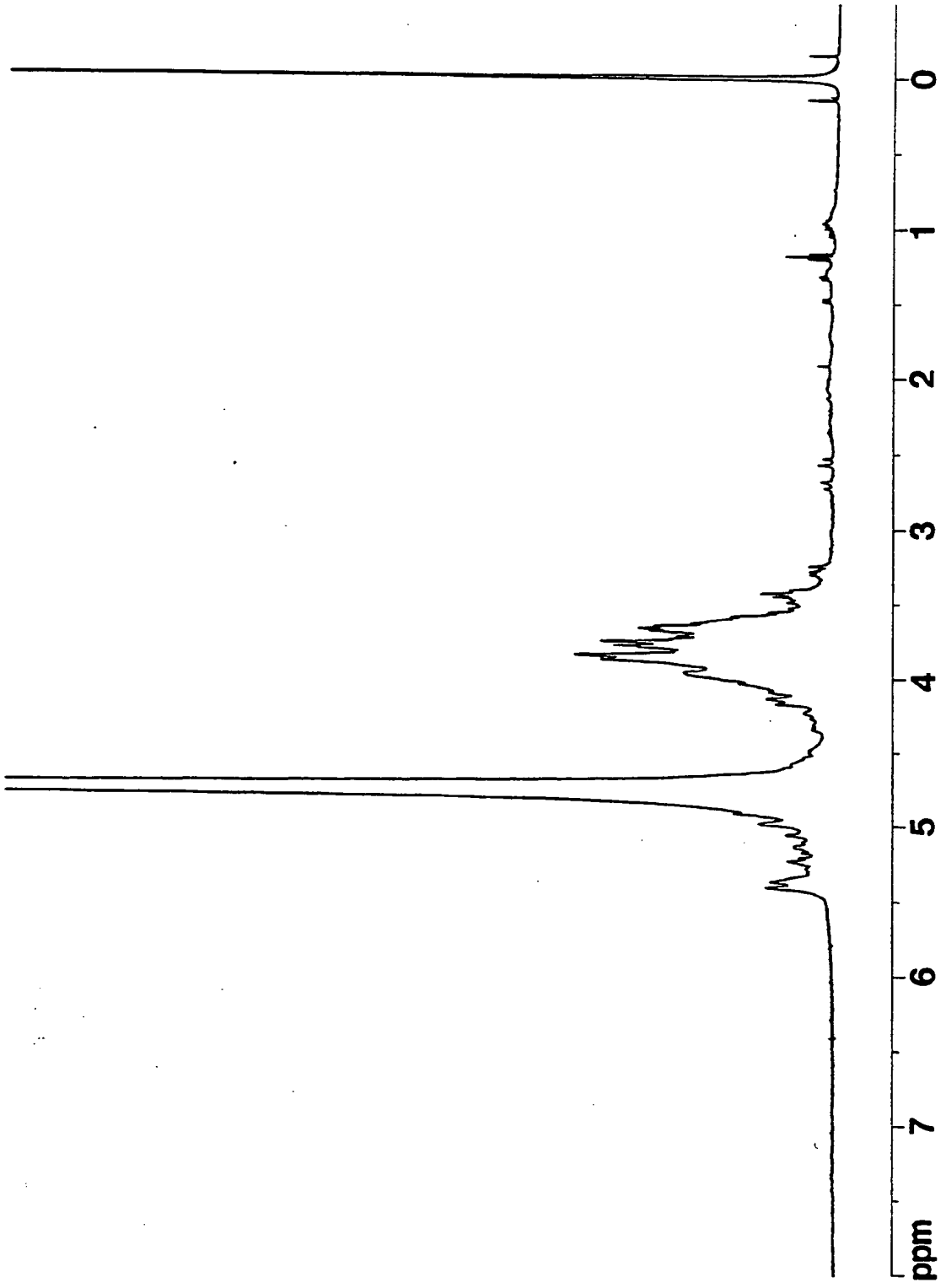
TM 7279/
32-m-1-5

Fig. 3



MG 7274/
9-18-1

Fig. 4



INTERNATIONAL SEARCH REPORT

Int onal Application No
PCT/EP 96/03535

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12P21/00 C12P19/04 A61K39/00 C08B37/00 C07K2/00
C12N1/16 //(C12N1/16,C12R1:725),(C12P21/00,C12R1:645),
(C12P19/04,C12R1:645)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12P C07K C08B A61K C12N C12R

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 93 07894 A (BOEHRINGER INGELHEIM VETMEDICA GMBH) 29 April 1993 cited in the application see the whole document ---	1-38

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 November 1996	18. 11. 96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Moreau, J

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

ernational application No.
PCT/EP 96/03535

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 30-31
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 30-31 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int ional Application No
PCT/EP 96/03535

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9307894	29-04-93	RU-C-	2020959	15-10-94
		CA-A-	2097702	22-04-93
		CZ-A-	9301448	19-01-94
		DE-U-	9218921	15-02-96
		EP-A-	0564620	13-10-93
		HU-A-	68503	28-06-95
		JP-T-	6506476	21-07-94
		PL-A-	299982	18-04-94
		PT-A-	100989	31-01-94
		SK-A-	71093	06-10-93